BEST AVAILABLE IMAGES

Defective images within this document are accurate representations of the original documents submitted by the applicant.

Defects in the images may include (but are not limited to):

- BLACK BORDERS
- TEXT CUT OFF AT TOP, BOTTOM OR SIDES
- FADED TEXT
- ILLEGIBLE TEXT
- SKEWED/SLANTED IMAGES
- COLORED PHOTOS
- BLACK OR VERY BLACK AND WHITE DARK PHOTOS
- GRAY SCALE DOCUMENTS

IMAGES ARE BEST AVAILABLE COPY.

As rescanning documents *will not* correct images, please do not report the images to the Image Problem Mailbox.

BUNDESREPUBLIK DEUTSCHLAND

EP00/1368

29. März 2000

Bescheinigung

09/913631

Die Theragene Biomedical Laboratories GmbH in Martinsried/Deutschland hat eine Patentanmeldung unter der Bezeichnung

"Hormone-Hormone Receptor Complexes and Nucleic Acid Constructs and Their Use in Gene Therapy"

am 19. Februar 1999 beim Deutschen Patent- und Markenamt eingereicht.

Die angehefteten Stücke sind eine richtige und genaue Wiedergabe der ursprünglichen Unterlagen dieser Patentanmeldung.

Die Anmeldung hat im Deutschen Patent- und Markenamt vorläufig die Symbole C 07 H, C 12 N und A 61 K der Internationalen Patentklassifikation erhalten.

München, den 20. März 2000

Deutsches Patent- und Markenamt

Der Präsident

Im Auftrag

Dzierzon

Aktenzeichen: 199 07 099.7

PRIORITY DOCUMENT

SUBMITTED OR TRANSMITTED IN COMPLIANCE WITH RULE 17.1(a) OR (b)

A 9161
06.90
11/98

4

Abstract of the Disclosure

The invention provides a composition of matter comprising a nucleotide sequence comprising at least one hormone responsive element (HRE), said hormone responsive element being coupled to a hormone-hormone receptor complex and further provides nucleic acid constructs comprising at least one hormone responsive element (HRE), wherein the hormone responsive element regulates a gene encoding a human blood-clotting factor, the invention having applications in gene therapy, and particularly, in the treatment of human blood clotting disorders, such as hemophilia.

Hormone –Hormone Receptor Complexes and Nucleic Acid Constructs and Their Use in Gene Therapy

Background of the Invention

1. Object of the Invention

The invention provides a composition of matter comprising a nucleotide sequence comprising at least one hormone responsive element (HRE), said hormone responsive element being coupled to a hormone-hormone receptor complex. It further relates to nucleic acid constructs comprising at least one hormone responsive element and vectors comprising such constructs, wherein the hormone responsive element regulates a gene encoding a human blood-clotting factor. The nucleic acid constructs, plasmids, and compositions of matter of the invention have applications in gene therapy, particularly in the treatment of human blood clotting disorders, such as hemophilia. They may also be used to up- or down-regulate target genes and for the delivery of vaccines.

2. Summary of the Related Art

Gene therapy is a method that holds great promise for many diseases and disorders. In general, it involves the transfer of recombinant genes or transgenes into somatic cells to replace proteins with a genetic defect or to interfere with the pathological process of an illness. In principle, gene therapy is a simple method. In practice, many disadvantages must still be overcome.

Research in gene therapy has concentrated on ways to most effectively incorporate DNA into cells of a patient. Viral vectors are currently the widely used vehicles in clinical gene therapy approaches. In terms of efficacy in gene expression, the

viral delivery systems have major advantages over techniques using DNA-lipid formulations as delivery vehicles or over mechanical methods, such as the gene gun. Although there are a variety of viral systems tested for gene therapeutical strategies, retroviral vectors and adenoviral vectors are presently the most widely used vehicles (Salmons, B. and Gunzburg, W. H., *Hum. Gene Ther.*, Vol. 4, 129, 1993; Kasahara, N. A., et al., *Science*, Vol. 266, 1373, 1994; Ali, M., et al., *Gene Ther.*, Vol. 1, 367, 1994.). Still, these systems have major disadvantages, such as potential viral contamination. Other safety concerns continue to hamper the development of clinical application of gene therapy using these viral systems. For example, recombinant retroviruses have the disadvantage of random chromosomal integration, which may lead to activation of oncogenes or inactivation of tumor-suppressor genes. Also, repetitive use of recombinant adenoviruses has caused severe immunological problems (Elkon, K. B. et al., *Proc. Natl. Acad. Sci. USA*, Vol. 94, 9814, 1997). The humoral response resulted in the production of antibodies to adenovirus proteins preventing subsequent infection. Immunosuppressive drugs may ameliorate these effects, but they place an additional burden on the patient (Dai, Y., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 92, 1401, 1995).

Yet another viral delivery system involves adenoassociated virus (AAV). The AAV requires coinfection with an unrelated helper virus. Although such recombinant AAV virions have proven useful for introducing several small gene sequences into host cells, gene delivery systems based on those particles are limited by the relative small size of AAV particles. This feature greatly reduces the range of appropriate gene protocols. Moreover, the need to also use a helper virus adds a complicating factor to this delivery system (Muzyczka, N., Curr. Top. Microbiol. Immunol., Vol. 158, 97, 1992).

Though safer, non-viral gene therapy approaches are also unsatisfactory. Problems with inefficient gene delivery or poor sustained expression are major drawbacks. Yet methods such as the direct injection of DNA into cellular compartments to mixtures of DNA with cationic lipids or polylysine allowing the transgene to cross the cell membrane more easily, have not overcome these hurdles (Felgner, P., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 84, 7413, 1987; Behr, J.-P., *Bioconjugate Chemistry*, Vol. 5, 382, 1994).

With the introduction of naked DNA (polynucleotide) sequences (including antisense DNA) into vertebrates, controlled expression of a protein is possible. The administration of the polynucleotide sequences is reported to be achieved by injection into tissues such as muscles, brain or skin or by introduction into the blood circulation (Wolff, J. A., et al., *Science*, Vol. 247, 1990; Lin, H., et al., *Circulation*, Vol. 82, 2217, 1990; Schwartz, B., et al., *Gene Ther.*, Vol. 3, 405, 1996). Also, a direct gene transfer into mammals has been reported for formulations of DNA encapsulated in liposomes and DNA entrapped in proteoliposomes containing receptor proteins. Although injected naked DNA leads to transgene expression, the efficiency is by far not comparable to viral-based DNA delivery systems. Nevertheless, naked DNA has the advantage of being without possible pathogenic effects. A limitation of the method of naked DNA injection is the fact that transgene expression is dose-dependent. The gene expression is saturable, and an increase in the amount of DNA injected leads to decreased protein production per plasmid. Thus, protein expression can dramatically decrease, if the amount of DNA injected is above a certain threshold.

Among the genetic disorders that the skilled artisan has sought to overcome using these prior art methods are those relating to blood clotting disorders, and in particular, hemophilia (Lozier, J. N. and Brinkhous, K. M., *JAMA*, Vol.271, 1994; Hoeben, R. C., *Biologicals*, Vol. 23, 27, 1995). For example, Hemophilia A and B are X-linked, recessive bleeding disorders caused by deficiencies of clotting Factors VIII and IX, respectively (Sadler, J. E. et al., in: *The Molecular Basis of Blood Diseases*, 575, 1987). The incidence of hemophilia is about 1 in 5,000 male births. Hemophiliacs suffer from excessive bleeding due to the lack of clotting at the site of wounds. The inability to clot properly causes damage to joints and internal tissues as well as posing risks to the proper treatment of cuts.

Treatment of Hemophilia A is possible by the administration of the blood clotting Factor VIII. Until recently, Factor VIII preparations had to be prepared by concentrating blood from donors, posing the risk of contamination by infectious agents, such as HIV and hepatitis. The gene for Factor VIII has been cloned (e.g., Vehar et al., *Nature* Vol. 312, 337 1984) allowing for the production of a recombinant product. Although recombinant methods provide Factor VIII of higher purity than blood concentrates, the

exogenous supply of Factor VIII to a patient still requires repeated doses throughout the lifetime of the patient, an inconvenient and expensive solution. Other forms of hemophilia include Hemophilia B, caused by a defect in the gene coding for Factor IX. The gene therapy systems described above have been attempted for the treatment of Hemophilia A and B with Factors VIII and IX, respectively. (See e.g., WO 94/29471). However, these systems have the disadvantages already discussed above.

The classical model of the action of hormones is based on the concept of binding interaction of the hormone to an intracellular receptor, located in the cytoplasm or the nucleus (Evans, R., *Science*, Vol. 240, 889, 1988). These intracellular receptors remain latent until exposed to their target hormone. When so exposed, the hormone receptor changes its conformation after the hormone is bound and translocates in the activated form into the cell nucleus where it binds as a dimer to hormone-responsive-elements (HRE) in the promoter region of hormone-regulated genes (Beato, M., *Cell*, Vol. 56, 335, 1989; O'Malley, B., et al., *Biol. Reprod.*, Vol. 46, 163, 1992). The HRE are enhancer elements usually located in the 5' flanking region of the specific hormone-induced gene.

An example of such intracellular receptors is the steroid receptor. Steroid receptors belong to a superfamily of ligand-dependent transcription factors characterized by a unique molecular structure. The centrally located highly conserved DNA-binding domain defines this superfamily. The second important and relatively invariant region is the COOH-terminal ligand-binding domain. An example of such a receptor is the progesterone receptor mediated by the steroid progesterone. At the progesterone receptor, progesterone acts as a natural agonist whereas it displays potent antimineralocorticoid properties both at the molecular and the systemic level. Besides classical effects on the uterus, antiepileptic, anxiolytic, hypnotic and anesthetic properties have been attributed to progesterone according to numerous studies.

Methods have been proposed for the use of mutant receptors, including mutant steroid receptors for gene therapy. For example, such methods are disclosed in WO 93/23431, WO 98/18925, WO 96/40911. WO 98/33903 discloses a genetic construct

comprising a steroid responsive element from a tissue specific gene, a coding sequence, and an SV40 enhancer.

Brief Description of the Invention

The object of the present invention is to overcome the disadvantages of the previous gene therapy delivery systems. The delivery system of the present invention is a composition of matter comprising a nucleic acid comprising at least one hormone responsive element (HRE), said hormone responsive element being coupled to a hormone-hormone receptor complex. A preferred embodiment of the composition of matter of the invention is one where the hormone responsive element is a steroid responsive element (SRE), and the receptor is a steroid receptor. Most preferably, the hormone responsive element is a progesterone responsive element (PRE), and the receptor is a progesterone receptor.

The present invention provides a delivery system for gene therapy that should overcome the prior art disadvantages. The presence of the hormone responsive element together with the nucleic acid encoding a gene of interest stimulates and enhances gene expression and, more importantly, encourages the binding of a hormone-hormone receptor complex. One hormone responsive element is preferably present as a nucleic acid dimer sequence or nucleic acid multimer sequence. Even an inverse orientation of the hormone responsive element will exert its proper function. The hormone-hormone receptor complex contains a hormone receptor that becomes activated after binding of its specific hormone. The hormone receptor in the activated state is able to recognize and bind to its specific hormone responsive element, which in the present invention is combined with a nucleic acid encoding the desired gene, e.g., a human blood-clotting factor.

Another aspect of the present invention is a nucleic acid construct comprising at least one hormone responsive element (HRE), and in particular, comprising at least one hormone responsive element for regulating the expression of a gene encoding a human blood clotting factor. A preferred embodiment is one where the hormone responsive

element is a steroid responsive element (SRE). Most preferably, the hormone responsive element is a progesterone responsive element (PRE).

Another aspect of the present invention are vectors comprising the nucleic acid constructs of the present invention. Embodiments of the invention further include transfected and transformed cells comprising these vectors and/or nucleic acids.

A further embodiment of the present invention is pharmaceutical compositions comprising a therapeutically effective dose of the nucleic acid constructs of the invention and a hormone. The hormone is preferably a steroid, and most preferably, progesterone. Another aspect of the invention is the use of the compositions of matter and nucleic acid constructs as medicaments against genetic disorders or diseases, such as hemophilia, as well as the use of the compositions of matter and nucleic acid constructs for the manufacture of a medicament against genetic disorders and diseases, such as hemophilia.

The invention further includes a method of introducing into a cell a nucleic acid construct encoding a gene of interest, e.g., a human blood-clotting factor, to express the gene of interest in the cell. This method incorporates a nucleic acid into the cell (via, for example, a vector) so that the cell expresses the gene encoded by the foreign nucleic acid. In this method, the nucleic acid encoding the gene, e.g., a human blood-clotting factor, is combined with a nucleic acid construct comprising at least one hormone responsive element (HRE), preferably a progesterone responsive element. The presence of the hormone responsive element together with the nucleic acid encoding the gene, e.g., a human blood-clotting factor, stimulates and enhances gene expression and encourages the binding of a hormone-hormone receptor complex in the target cell.

Another aspect of the invention is a method of treating a blood clotting disorder by administering a therapeutically effective amount of the composition of matter of the invention to an organism.

According to the method of the invention vaccination is another aspect of the embodiment. Introducing a nucleic acid construct or composition of matter of the invention comprising a gene for an antigen or containing viral sequence into a cell (DNA

or mRNA vaccines) using the method mentioned above may also provide a way to stimulate the cellular immune response.

Brief Description of the Drawings

Figure 1 is a diagram of the vector pTGFG1.

Figure 2 is a diagram of the vector pTGFG4.

Figure 3 is a diagram of the vector pTGFG5.

Figure 4 is a diagram of the vector pTGFG6.

Figure 5 is a diagram of the vector pTGFG7.

Figure 6 is a diagram of the vector pTGFG8.

Figure 7 is a diagram of the vector pTGFG9.

Figure 8 is a diagram of the vector pTGFG10.

Figure 9 is a diagram of the vector pTGFG11.

Figure 10 is a diagram of the vector pTGFG13.

Figure 11 is a diagram of the vector pTGFG14.

Figure 12 is a diagram of the vector pTGFG15.

Figure 13 is a diagram of the vector pTGFG16.

Figure 14 is a diagram of the vector pTGFG18.

Figure 15 is a diagram of the vector pTGFG19.

Figure 16 is a diagram of the vector pTGFG20.

Figure 17 is a diagram of the vector pTGFG21.

Figure 18 is a diagram of the vector pTGFG22.

Figure 19 is a diagram of the vector pTGFG23.

Figure 20 is a diagram of the vector pTGFG24.

Figure 21 is a diagram of the vector pTGFG25.

Figure 22 is a diagram of the vector pTGFG26.

Figure 23 is a diagram of the vector pTGFG27.

Figure 24 is a diagram of the vector pTGFG28.

Figure 25 is a diagram of the vector pTGFG29.

Figure 26 is a diagram of the vector pTGFG30.

Figure 27 is a diagram of the vector pTGFG31.

Figure 28 is a diagram of the vector pTGFG32.

Figure 29 is a diagram of the vector pTGFG33.

Figure 30 is a diagram of the vector pTGFG2.

Figure 31 is a diagram of the vector pTGFG34.

Figure 32 is a diagram of the vector pTGFG35.

Figure 33 is a diagram of the vector pTGFG36.

Figure 34 is a diagram of the vector pTGFG37.

Figure 35 is a diagram of the vector pTGFG38.

Figure 36 is a diagram of the vector pTGFG53.

Figure 37 is a diagram of the vector pTGFG64.

Figure 38 is a diagram of the vector pTGFG0.

Figure 39 is a diagram of the vector pTGFG66.

Figure 40 is a diagram of the vector pTGFG67.

Figure 41 is a diagram of the vector pTGFG68.

Figure 42 is a diagram of the vector pTGFG69.

Figure 43 is a diagram of the vector pTGFG82.

Figure 44 is a diagram of the vector pTGFG95.

Figure 45 is the DNA sequence of vector pTGFG36 (SEQ ID No. 1).

Figure 46 is the DNA sequence of vector pTGFG67 (SEQ ID No. 2).

Figure 47 shows a GFP concentration curve for cell homogenates after transfection with pTGFG5 and pTGFG20, respectively.

Figure 48 shows corresponding light (a and c) and fluorescent (b and d) micrographs of HeLa cells transfected with pTGFG5 (a and b) and pTGFG20 (c and d), respectively.

Detailed Description of the Invention

1. Definitions

"Nucleic acid" means DNA, cDNA, mRNA, tRNA. The nucleic acid may be linear or circular, double-stranded or single-stranded.

"Nucleic acid construct" refers to a composite of nucleic acid elements in relation to one another. The nucleic acid elements of the construct may be incorporated into a

vector in such an orientation that a desired gene may be transcribed, and if desired, a desired protein may be expressed.

"Hormone responsive element" (HRE) refers to regions of nucleic acids, and in particular, DNA, which regulate transcription of genes in response to hormone activation. HREs are typically about 10-40 nucleotides in length, and more usually, about 13-20 nucleotides in length. As explained above, HREs become activated when a hormone binds to its corresponding intracellular receptor causing a conformational change, so that the receptor has increased affinity for the HRE and binds to it. The HRE, in turn, stimulates transcription. A "steroid responsive element" (SRE) is an HRE that regulates transcription of genes in response to steroid activation. A "progesterone responsive element" (PRE) is an HRE/SRE that regulates transcription of genes in response to progesterone activation.

A "hormone receptor" refers to a receptor which binds to or is activated by a hormone. A "steroid receptor" refers to a receptor which binds to or is activated by a steroid hormone. A "progesterone receptor" is a receptor which binds to or is activated by the steroid hormone progesterone.

"Gene" refers to DNA involved in expressing a polypeptide, optionally including leader and trailer sequences and introns and exons.

"Vector" refers to any genetic construct, such as a plasmid, phage, cosmid, etc., which is capable of replication when associated with the proper control elements and which can transfer gene sequences between cells. The term includes cloning and expression vehicles.

"Promoter" refers to a region of regulatory DNA sequences for the control of transcription of a gene to which RNA polymerase binds. The promoter forms an initiation complex with RNA polymerase to initiate and drive transcription activity. "Enhancers" may activate the complex or "silencers" may inhibit the complex. A "tissue-specific promoter" is a promoter found in the DNA of tissue for transcription of genes expressed in specific tissue.

"Therapeutically effective dose" of the products of the invention refers to a dose effective for treatment or prophylaxis, for example, a dose that yields effective treatment or reduction of the symptoms of hemophilia. It is also a dose that measurably activates expression of a target gene as determined by measurements of target protein levels, or a dose that is predictable to be effective for treatment or prophylaxis by extrapolating from *in vitro* or *in vivo* data. The determination of a therapeutically effective dose is within the purview of one skilled in the art.

"Encodes" or "encoding" refers to a nucleic acid sequence transcribed (in case of DNA) or translated (in the case of mRNA) into a polypeptide *in vitro* or *in vivo* when placed under the control of appropriate regulatory sequences.

For the purposes of this application, "express", "expressing" or "expression" shall refer to transcription and translation of a gene encoding a protein.

2. Detailed Description and Examples

As stated above, an object of the present invention is to provide a new and improved delivery system for gene therapy. The invention thus provides in one embodiment a composition of matter comprising a nucleic acid comprising at least one hormone responsive element (HRE), said hormone responsive element being coupled to a hormone-hormone receptor complex. A preferred embodiment of the composition of matter of the invention is one where the hormone responsive element is a steroid responsive element (SRE), and the receptor is a steroid receptor. Most preferably, the hormone responsive element is a progesterone responsive element (PRE), and the receptor is a progesterone receptor.

Potential HREs for use in the present invention have been previously described. For example, GREs (Scheidereit, C., et al., *Nature,* Vol. 304, 749, 1983; von der Ahe, D., et al., *Proc. Natl. Acad. Sci. USA*, Vol. 83, 2817, 1986), EREs or PREs (Chambon, P., et al., *Rec. Prog. Horm. Res.*, Vol., 40, 1, 1984; Klock, G., et al., *Nature,* Vol. 329, 734, 1987). As already stated above, the most preferred HRE for the invention is a PRE.

Specifically, the preferred PRE is described in Example I. The nucleic acid for use in the invention comprises at least one hormone responsive element. Preferred is a nucleic acid comprising one HRE, but it may comprise more than one HRE. For example, the nucleic acid may comprise three or five HREs. The most preferred embodiment is a nucleic acid comprising one PRE.

Potential hormone receptors for use in the present invention are, for example, estrogen receptors, mineralocorticoid receptors, glucocorticoid receptors, retinoic acid receptors, androgen, calcitriol, thyroid hormone or progesterone receptors and orphan receptors. Such receptors have been previously described. (Green, S., et al., *Nature,* Vol. 320, 134, 1986; Green, G. L.,et al., *Science*, Vol. 231, 1150, 1986; Arriza, J. L., et al., *Science*, Vol. 237, 268, 1987; Hollenberg, S. M., et al., *Nature*, Vol. 318, 635, 1985; Petkovitch, M., et al., *Nature*, Vol. 330, 444, 1987; Giguere, V., et al., *Nature* , Vol. 330, 624, 1987; Tilley, W., et al., *Proc. Natl. Acad. Sci. USA,* Vol. 86, 327, 1989; Baker, A. R., et al., *Proc. Natl. Acad. Sci. USA,* Vol. 85, 3294, 1988; Weinberger, C., et al., *Nature*, Vol. 324, 641, 1986; Sap, J., et al., *Nature*, Vol. 324, 635, 1086; Misrahi, M., et al., *Biochem. Biophys. Res. Commun.,* Vol. 143, 740, 1987; Kastner, P., et al., *Cell*, Vol. 83, 859, 1995). These receptors may be from human or other mammalian sources, although human is preferred. Nucleotide and/or amino acid sequences of human steroid receptors are available in the GenBank: mineralocorticoid receptor: M16801; glucocorticoid receptor α: M10901; glucocorticoid receptor α_2: U01351; glucocorticoid receptor β: M11050; retinoic acid receptor α: AF088888 (exon 1), AF088889 (exon 2), AF088890 (exon 3), AF088891 (exon 4), AF088892 (exon 5 and 6), AF088893 (exon 7), AF088894 (exon 8), AF088895 (exon 9 and complete cDNA); retinoic acid receptor γ: M24857; androgen receptor: M27423 (exon 1), M27424 (exon 2), M27425 (exon 3), M27436 (exon 4), M27427 (exon 5), M27428 (exon 6), M27429 (exon 7), M27430 (exon 8); thyroid hormone receptor α_1: M24748, thyroid hormone receptor α_2: J03239; progesterone receptor: AF016381; somatotropin receptor: J00148; vitamin D receptor (calcitriol receptor): J03258.

The skilled person will understand that expression of the receptor proteins can be achieved by standard methods, e.g. via PCR-cloning of the known cDNAs from cDNA libraries and overexpression of the corresponding proteins in suitable expression vectors,

such as, for example, the vectors of the present invention, in suitable host cells, e.g., COS cells. Accordingly, subsequent purification of the cytosolic fraction can be achieved by routine methods such as affinity chromatography purification. For this purpose, various suitable antibodies against the desired receptor are commercially available. For example, polyclonal antibodies against the mouse progesterone receptor that have a sufficiently high cross-reactivity for the human protein are available from Dianova (Hamburg, Germany). Likewise, further purification can be achieved by standard methods, e.g., chromatographical methods such as ion-exchange chromatography and/or FPLC.

The most preferred receptor is the progesterone receptor. Preferably, the receptor is a human progesterone receptor. Such a human progesterone receptor (from T47D human breast cancer cells) is disclosed in US Patent No. 4,742,000, and cells expressing this receptor have been deposited (ATCC deposit number HTB, 133). As already described above, it would be routine to purify such a receptor from the cytosol using receptor specific antibodies. In addition, US Patent No. 4,742,000 discloses a method for purification of the human progesterone receptor using a specific steroid affinity resin (cf. Grandics et al., Endocrinology, Vol. 110, 1088, 1982). Briefly, the cytosolic fraction of the T47D cells is passed over Sterogel, a commercial preparation of deoxycorticosterone coupled to Sepharose 2B that selectively binds the progesterone receptor. After washing with loading buffer, the bound receptor is eluted with a buffer containing progesterone. The eluted steroid-receptor complex is then chromatographed on DEAE-Biogel and eluted stepwise with a buffer containing 0.2M NaCl. Subsequently, the bound progesterone can be readily exchanged. As described above, further purification can be achieved by routine methods well-known to the skilled person.

Mutated versions of these receptors and derivatives thereof, that still retain the function of the receptors to bind a ligand and thereby become activated and bind DNA and regulate transcription, may also be employed in the invention. Such derivative may be a chemical derivative, variant, chimera, hybrid, analog, or fusion.

The hormone in the composition of matter may include synthetic and natural hormones, such as estrogen, testosterone, glucocorticoid, androgen, thyroid hormone, and progesterone or derivatives thereof. These are widely available. Progesterone is most preferred. For example, natural micronized progesterone has been marketed in France under the trademark of UTROGESTAN® since 1980. Its properties are similar to the endogenous progesterone, in particular, it has antiestrogen, gestagen, slightly antiandrogen and antimineralocorticoid properties.

Micronized progesterone has advantages that make it a suitable carrier for genes or nucleic acid constructs to target cells. Specifically, the synergistic effect of the double process of micronization and suspension in long-chain fatty acids results in increasing progesterone absorption. It has been demonstrated that after oral administration of 100 mg of UTROGESTAN®, peak plasma progesterone levels were obtained after 1-4 hours in most cases (Padwick, M. L., et al., *Fertil. Steril.*, Vol. 46, 402, 1986). Later on, the levels declined substantially, although they were still elevated at 12 hours. Even at 84 hours the levels were slightly higher than baseline. A U.S. kinetic study confirmed earlier work demonstrating the bioavailability of oral micronized progesterone. They showed a peak effect at 2 hours followed by rapid decrease in plasma progesterone level (Simon, J. A., et al., *Fertil., Steril.*, Vol., 60, 26, 1993).

A further advantage of using progesterone as a carrier is the low level of disadvantageous side effects. Orally administered progesterone adversely affects neither plasma lipids (Jensen, J. et al., Am. J. Obstet. Gynecol., Vol. 156, 66, 1987) nor carbohydrate metabolism (Mosnier-Pudar, H. et al., Arch. Mal. Coeur, Vol 84, 1111, 1991). Further, progesterone does not affect liver enzymes (ASAT, ALAT, AFOS), sex-hormone binding-globulin (SHBG) synthesis or HDL-cholesterol levels at daily doses of 200 mg and 300 mg. Although the plasma levels of deoxycorticosterone may increase substantially during UTROGESTAN® treatment, there are strong indications that the mineralocorticoid effects of this progesterone metabolite are completely counteracted by the anti-mineralocorticoid effects of progesterone itself. This is apparent from a comparative study (Corvol, P., et al., In: Progesterone and progestins. Raven Press, New York, 179, 1983) in which oral UTROGESTAN® was capable of antagonizing the mineralocorticoid effects of 9-alpha-fluorohydrocortisone.

The skilled artisan will appreciate that the composition may contain other components capable of assisting in introducing the nucleic acid into a cell for the purpose of gene therapy. Specifically, the composition may further contain ß-cyclodextrine, glycerin, lecithin or corn oil. For example, the composition of hormone-hormone receptor nucleic acid complex of the invention may be provided orally to humans or animals as a gelatin capsule. Progesterone therein could be present in a concentration of 200 –300 mg dissolved in a 35 % or 40 % β-cyclodextrin solution or in cornoil or gycerol with peanut oil together with lecithin.

Another aspect of the present invention is a nucleic acid construct comprising at least one hormone responsive element (HRE), and in particular, comprising at least one hormone responsive element for regulating the expression of a gene, such as, for example, a gene encoding a human blood clotting factor. A preferred embodiment is one where the hormone responsive element is a steroid responsive element (SRE). Most preferably, the hormone responsive element is a progesterone responsive element (PRE). This nucleic acid construct may be used as the nucleic acid in the composition of matter of the first aspect of the invention.

Aside from the HREs, SREs, or PREs already disclosed above, the nucleic acid in the present invention may further contain promoter, enhancer, or silencer sequences. The promoter may be ubiquitous or tissue-specific. Of the ubiquitous promoters, the CMV promoter is most preferred. However, a tissue-specific promoter is preferred over a ubiquitous promoter. For example, the tissue-specific promoters envisioned for the instant invention include alpha$_1$-antitrypsin. The nucleic acid construct may further comprise additional sequences such as the ampicillin resistance gene. Other reporter sequences known to the skilled artisan may also be included, such as, for example, the green fluorescent protein (GFP), luciferase, ß-galactosidase or chloramphenicyl-transferase (CAT). As an enhancer sequence, the SV40 intron and SV40 Poly A are most preferred. A preferred nucleic acid construct contains sequentially from the 5' to the 3' end: a PRE, a CMV promoter, a gene of interest, SV40 Intron and SV40 poly A enhancer sequence, and an ampicillin resistant gene.

The gene of interest may be chosen from those encoding proteins lacking in a variety of genetic disorders or involved in conditions related to inappropriate responses to hormones, for example, hormone-dependent cancers such as breast, ovarian, and endometrial cancers and prostrate cancer. The gene of interest may also be used to replace a defective gene resulting in such genetic disorders as hemophilia, von Willebrand disease, and cystic fibrosis. The gene of interest includes mutations of such gene or a gene encoding a fusion product. The nucleic acid construct of the present invention may comprise more than one gene of interest.

In particular, the gene of interest may replace genes for a blood clotting factor, and preferably a human blood-clotting factor. The genes encoding Factor VIII and Factor IX, involved in Hemophilia A and B, respectively, are good candidates for the invention. Other candidates include the gene encoding von Willebrand factor, Factor IV, Factor X, or protein C.

Other useful genes include, but are not limited to, hormone genes such as the genes encoding for insulin, parathyroid hormone, luteinizing hormone releasing factor (LHRH), alpha and beta seminal inhibins and human growth hormone; hormone receptor genes such as the glucocorticoid receptor, the estrogen receptor, the progesterone receptor, the retinoic acid receptor; growth factors such as vascular endothelial growth factor (VEGF), nerve growth factor, epidermal growth factor; enzyme genes; genes encoding cytokines or lymphokines such as interferons, granulocytic macrophage colony stimulating factor (GM-CSF), colony stimulating factor-1 (CSF-1), tumor necrosis factor (TNF), and erythropoietin (EPO); genes encoding inhibitor substances such as $alpha_1$-antitrypsin, and genes encoding substances that function as drugs, e. g., genes encoding the diphteria and cholera toxins, ricin or cobra venom factor. Also, antisense sequences may be administered as genetic material.

Another aspect of the present invention is vectors comprising the nucleic acid constructs of the present invention. These vectors may be used in the composition matter of the present invention. Preferably, however, the nucleic acid sequence for use in the invention is linear rather than circular. The vectors may be capable of expressing

the nucleic acid in the nucleic acid construct transiently, permanently, or episomally. As noted above, the nucleic acid construct therein may further contain additional elements.

Embodiments of the invention further include transfected and transformed cells comprising these vectors and/or nucleic acid constructs. Within the scope of this invention, a transfected cell is one in which foreign DNA has been incorporated. Methods of transfection may include microinjection, $CaPO_4$ precipitation, electroporation, liposome fusion, or by gene gun. Most preferably, transfection is achieved by electroporation.

Transformation refers to introducing genetic material into a cell, such as the vectors or nucleic acid constructs of the invention, rendering the cell transiently, stably, or permanently altered so that the cell expresses a specific gene product or is otherwise altered in its expression. Transformation may be achieved by *in vivo* or *in vitro* techniques, although *in vivo* transformation is preferred.

A further embodiment of the present invention is pharmaceutical compositions comprising a therapeutically effective dose of the nucleic acid constructs of the invention and a hormone. The hormone is preferably a steroid, and most preferably, progesterone, as described above. The dose is dependent on the condition to be treated, the characteristics of the patient, and the result sought to be achieved. Determining dosage is within the realm of the skilled artisan.

The pharmaceutical composition (or, alternatively, the composition of matter, the nucleic acid construct, or the vector) of the present invention may be administered orally, intravenously, intramuscularly, subcutaneously, topically, or by gene gun. Oral administration with micronized hormone is preferred. Delivery may be systemic or directed at certain tissue.

The invention further includes a method of introducing into a cell a nucleic acid construct encoding a gene of interest, e.g., a human blood-clotting factor, to express the blood-clotting factor in the cell. In this method, the nucleic acid encoding a human blood-clotting factor is combined with a nucleic acid construct comprising at least one hormone responsive element (HRE), preferably a progesterone responsive element.

The mixture of nucleic acid bound to the hormone-hormone receptor complex together with an excess of hormone, preferably progesterone, will be used to introduce the nucleic acid into a cell by various methods known to the skilled artisan and outlined above. The cell-uptake will be stimulated by the interaction of the hormone at the cell membrane level. The hormone or steroid interacts with the lipid bilayer of the cell membrane not only through membrane perturbation but also through activation of certain hormone- or steroid-sensitive membrane receptors. This has been demonstrated for progesterone and other steroids. Last but not least, it is known that hormones are able to cross the cell membrane by diffusion. In the present invention, the nucleic acid bound to the hormone-hormone receptor complex should be transported through the membrane during the process of diffusion.

Another aspect of the invention is a method of treating a blood clotting disorder by administering a therapeutically effective amount of the composition of matter of the invention to an organism. This method involves the administration and dosage considerations already discussed.

Experiments have been performed to illustrate the technical aspects of the present invention. These experiments are described in Examples II-IV.

The following are examples of the present invention. The skilled artisan will be readily recognize that the invention is not limited to these examples.

Example I: Construction of Vectors

Production of the vector pTGFG1

The vector pUC19 (MBI Fermentas) was digested with XbaI, treated with Klenow-Enzyme and religated. This XbaI deleted vector was then digested with EcoRI, treated with Klenow-Enzyme and religated in order to delete the EcoRI site. For insertion of a XbaI site in the SacI site of this vector it was digested with SacI, treated

with T4-Polymerase, dephosphorylated with Alkaline Phosphatase and ligated with the XbaI-linker CTCTAGAG (Biolabs #1032). Another XbaI-site was inserted by digesting the newly produced vector with HindIII, treating it with Klenow, dephosphorylating it with Alkaline Phosphatase and ligating it with the XbaI-linker CTCTAGAG (Biolabs #1032). This vector was named pUC19/X.

In order to destroy the XbaI-site present in the vector phGFP-S65T (Clontech) this vector was digested with XbaI, treated with Klenow-Enzyme and religated resulting in the vector pGFP/0. A 2.3 kb fragment containing the GFP-Gene was isolated after digesting pGFP/0 with MluI, treating it with Klenow-Enzyme and digesting it with BamHI. This fragment was inserted into the multiple cloning site of the vector pUC19/X which was digested with SalI, treated with Klenow-Enzyme and digested with BamHI. The resulting vector was named pTGFG1 (Figure 1).

Production of the insert PRE(ds)

The Oligonucleotides (Metabion) PRE-S (5'-GGG GTA CCA GCT TCG TAG CTA GAA CAT CAT GTT CTG GGA TAT CAG CTT CGT AGC TAG AAC ATC ATG TTC TGG TAC CCC-3') (SEQ ID No. 3) and PRE-AS (5'-GGG GTA CCA GAA CAT GAT GTT CTA GCT ACG AAG CTG ATA TCC CAG AAC ATG ATG TTC TAG CTA CGA AGC TGG TAC CCC-3') (SEQ. ID No. 4) were hybridized and phosphorylated by kinase reaction, resulting in the inserts PRE(ds).

Production of the vector pTGFG4

The vector pTGFG1 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG4 (Figure 2).

Production of the vector pTGFG5

The vector pTGFG1 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG5 (Figure 3).

Production of the vector pTGFG6

The vector pTGFG1 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG6 (Figure 4).

Production of the vector pTGFG7

The vector pTGFG1 was digested with KpnI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG7 (Figure 5).

Production of the vector pTGFG8

The vector pTGFG7 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG8 (Figure 6).

Production of the vector pTGFG9

The vector pTGFG7 was digested with SapI, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG9 (Figure 7).

Production of the vector pTGFG10

The vector pTGFG7 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG10 (Figure 8).

Production of the vector pTGFG11

The vector pTGFG7 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG11. (Figure 9).

Production of the vector pTGFG13

The vector pTGFG6 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG13 (Figure 10).

Production of the vector pTGFG14

The vector pTGFG6 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG14 (Figure 11).

Production of the vector pTGFG15

The vector pTGFG5 was digested with SapI, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG15 (Figure12).

Production of the vector pTGFG16

The vector pTGFG5 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG16 (Figure 13).

Production of the vector pTGFG18

The vector pTGFG9 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG18 (Figure 14).

Production of the vector pTGFG19

The vector pTGFG10 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG19 (Figure 15).

Production of the vector pTGFG20

The vector pTGFG11 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG20 (Figure 16).

Production of the vector pTGFG21

The vector pTGFG15 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG21 (Figure 17).

Production of the vector pTGFG22

The vector pTGFG14 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG22 (Figure 18).

Production of the vector pTGFG23

The vector pTGFG11 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG23 (Figure 19).

Production of the vector pTGFG24

The vector pTGFG9 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG24 (Figure 20).

Production of the vector pTGFG25

The vector pTGFG14 was digested with SapI, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG25 (Figure 21).

Production of the vector pTGFG26

The vector pTGFG16 was digested with SapI, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG26 (Figure 22).

Production of the vector pTGFG27

The vector pTGFG9 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG27 (Figure 23).

Production of the vector pTGFG28

The vector pTGFG18 was digested with EheI and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG28 (Figure 24).

Production of the vector pTGFG29

The vector pTGFG25 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG29 (Figure 25).

Production of the vector pTGFG30

The vector pTGFG27 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG30 (Figure 26).

Production of the vector pTGFG31

The vector pTGFG24 was digested with EcoO109I, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG31 (Figure 27).

Production of the vector pTGFG32

The vector pTGFG19 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG32 (Figure 28).

Production of the vector pTGFG33

The vector pTGFG28 was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. It was then ligated with the PRE(ds) insert, resulting in the vector pTGFG33 (Figure 29).

Production of the vector pTGFG2

The vector pUC19 (MBI Fermentas) was digested with SalI, treated with Klenow-Enzyme and dephosphorylated with Alkaline Phosphatase. It was ligated to the NotI-linker GCGGCCGC (Biolabs # 1045), resulting in the vector pUC19/N.

A 1.4 kb fragment containing the open reading frame of the human clotting factor IX, isolated from a human cDNA library, was inserted into the PstI-site of the vector pUC19/N which was digested with PstI, treated with T4-Polymerase and dephosphorylated with Alkaline Phosphatase. From the resulting vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG1. The resulting vector was named pTGFG2 (Figure 30).

Production of the vector pTGFG34

From the vector pUC19/N-FIX, a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with HindIII and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG3 resulting in the vector pTGFG34 (Figure 31).

Production of the vector pTGFG35

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with HindIII and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG4 resulting in the vector pTGFG35 (Figure 32).

Production of the vector pTGFG36

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG5 resulting in the vector pTGFG36 (Figure 33). This vector is a preferred one for delivery of Factor IX into the cell, and its DNA sequence is provided in Figure 46.

Production of the vector pTGFG37

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with HindIII and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG6 resulting in the vector pTGFG37 (Figure 35).

Production of the vector pTGFG38

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG7 resulting in the vector pTGFG38 (Figure 35).

Production of the vector pTGFG53

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG20 resulting in the vector pTGFG53 (Figure 36).

Production of the vector pTGFG64

From the vector pUC19/N-FIX a 1.4 kb fragment containing the open reading frame of the human clotting factor IX was cut out by double-digestion with Hind III and NotI. This fragment was ligated to the 4.3 kb fragment of the HindIII and NotI double-digested vector pTGFG33 resulting in the vector pTGFG64 (Figure 37).

Production of the insert ALLG(ds)

The Oligonucleotides (Metabion) ALLG1/1 (5'-AGC TTG ACC TCG AGC AAG C-3') (SEQ. ID NO: 6) and ALLG2 (5'-GGC CGC TTG CTC GAG GTC A-3') (SEQ. ID NO: 7) were hybridized and phosphorylated by kinase reaction, resulting in the inserts ALLG(ds). The insert ALLG (ds) was constructed to introduce into the vector of choice a sequence with a multiple cloning site for the possible introduction of other transgenes.

Production of the vector pTGFG0

The vector pTGFG1 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG0 (Figure 38).

Production of the vector pTGFG66

The vector pTGFG4 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG66 (Figure 39).

Production of the vector pTGFG67

The vector pTGFG5 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG67 (Figure 40). This vector is preferred for the delivery of any gene inserted into the cloning site, and its DNA sequence is provided in Figure 47.

Production of the vector pTGFG68

The vector pTGFG6 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG68 (Figure 41).

Production of the vector pTGFG69

The vector pTGFG7 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG69 (Figure 42).

Production of the vector pTGFG82

The vector pTGFG20 was double-digested with HindIII and NotI. The 4.3 kb fragment was ligated with the insert ALLG(ds), resulting in the vector pTGFG82 (Figure 43).

Production of the vector pTGFG95

The vector pTGFG33 was double-digested with HindIII and NotI. The 4.3 kb fragment was litigated with the insert ALLG(ds), resulting in the vector pTGFG95. (Figure 44)

Example II: Isolation of Human Factor IX cDNA

Factor IX cDNA was amplified from human liver cDNA (Clontech) using two primers overlapping the start and termination codon of the factor IX open reading frame resulting in a 1387 bp fragment containing the entire open reading frame. Restriction sites for EcoRI (upstream) and BamHI (downstream) were included at the end of each primer to facilitate cloning. Amplification was performed with Pwo Polymerase (Boehringer Mannheim) in 50 µl reaction volume [10 mM Tris HCl pH 8.85, 25 mM KCl, 5 mM $(NH_4)_2SO_4$, 2 mM $MgSO_4$] with 30 incubation cycles 96° C 1 min., 60° C 1 min., 72° C 2 min., followed by a final extension step at 72° C 10 min.

Reaction products were ligated into the EcoRI- and BamHI-sites of pUC19 and transformed into *E. coli* DH5-a. Positive clones were selected. Sequences were confirmed by cycle sequencing (Amersham) from both ends with labeled primers (IR-700) and automated analysis on the LiCor sequencing system (MWG, Biotech).

The following primers were used :

gGAATTCcgcaaaggttATGCAGCGCGTGAACATGATCATGGC(upstream)(SEQ. ID NO:8)

cgcGGATCCATTAAGTGAGCTTTGTTTTTCCTTAATCC (downstream)(SEQ. ID NO:9)

Example III: Expression and Quantification of the Marker Protein GFP ("Green Fluorescent Protein")

HeLa cells were transfected by electroporation with plasmids pTGFG5 or pTGFG20. Transfected cells were harvested, and the cell pellets were homogenized and lysated in a buffer containing phosphate-buffered saline (pH 7.5) and 10 mM PMSF. The concentration of green fluorescent protein (GFP) in the cell homogenate was determined by competitive ELISA.

For this purpose, GFP was coated in a defined concentration on microtiter plates. Then, GFP samples were added in presence of anti-GFP antibody. After several washing steps, a labeled secondary antibody was added in order to trace the first antibody. The colorimetric reaction was measured photometrically (extinction). Generally, the more GFP was added, the less antibody was left to bind the coated GFP. Thus, reduction of extinction corresponded to higher GFP concentration in the sample.

A concentration curve of GFP was determined by linear regression (Figure 47) using bovine serum albumin (BSA) as a reference. A mean value of 2.4 µg GFP/ml for pTGFG5 (1 PRE) and 5.2 µg GFP/ml for pTGFG20 (3PREs) was found.

Figures 48 a-d show micrographs of HeLa cell cultures transfected with pTGFG5 (Fig. 48 a and b) and pTGFG20 (Fig. 48 c and d), respectively. Figures 46 a and c represent light microscopic views as controls, and Fig. 48 b and d show the corresponding cell patches in the fluorescent mode. Routinely, more than 50% of the cells expressed GFP, indicating very efficient expression, the presence of only one PRE showing more efficient expression.

Example IV: Human Factor IX Quantification by ELISA Assay

HeLa cells were transfected either by electroporation or using liposome reagent DOTAP (Boehringer Mannheim) with plasmids pTGFG36, pTGFG53 and pTGFG64. These plasmids contain the cDNA of human clotting factor IX. Recombinant human factor IX was secreted into the supernatant of the cell culture and quantified using a sandwich ELISA method.

0.11 M sodium citrate and 10 mM PMSF were added in order to prevent degradation of human factor IX. The enzyme-immunological in vitro assay "Asserachrom IX:AG" from Boehringer-Mannheim was used in order to determine the concentration of expressed human clotting factor IX. The factor IX-standard from Octapharma AG was used as a standard in aqueous solutions of 28 IU/ml.

In six different transfection experiments, in which HeLa cells with plasmids containing human factor IX-cDNA (pTGFG36, 53 and 64) were transfected using either electroporation or lipid-transfection reagent (DOTAP, Boehringer Mannheim), a concentration range of 3-25 ng/ml human clotting factor IX was reached.

Claims:

1. A composition of matter comprising a nucleic acid comprising a hormone responsive element (HRE), said hormone responsive element being coupled to a hormone-hormone receptor complex.

2. The composition of matter of claim 1, wherein the nucleic acid further comprises a gene encoding a blood clotting factor.

3. The composition of matter of claim 2, wherein the human blood clotting factor is selected from the group consisting of Factor VIII, Factor IX, and von Willebrand Factor (vWF).

4. The composition of matter of claim 2, wherein the hormone responsive element is a steroid responsive element (SRE).

5. The composition of matter of claim 4, wherein the steroid responsive element (SRE) is a progesterone responsive element (PRE).

6. The composition of matter of claim 1, wherein the complex is a steroid-steroid receptor complex.

7. The composition of matter of claim 6, wherein the receptor is a progesterone receptor and the steroid is progesterone.

8. The composition of matter of claim 3, where the human blood clotting factor is Factor IX.

9. The composition of matter of claim 7, where the human blood clotting factor is Factor IX.

10. A nucleic acid construct comprising at least one hormone responsive element (HRE) for regulating the expression of a gene encoding a human blood clotting factor.

11. The nucleic acid construct of claim 10, wherein the hormone responsive element is a steroid responsive element (SRE).

12. The nucleic acid construct of claim 11, wherein the steroid responsive element is a progesterone responsive element (PRE).

13. The nucleic acid construct of claim 10, wherein the human blood clotting factor is selected from the group consisting of Factor VIII, Factor IX, and von Willebrand Factor (vWF).

14. The nucleic acid construct of claim 10, which further comprises a tissue-specific promoter.

15. The nucleic acid construct of claim 10, wherein the hormone responsive element (HRE) is a progesterone responsive element (PRE) and the blood clotting factor is Factor IX.

16. The nucleic acid construct of claim 15, which further comprises a tissue-specific promoter.

17. A vector comprising the nucleic acid construct of claim 10.

18. A vector comprising the nucleic acid construct of claim 11.

19. A vector comprising the nucleic acid construct of claim 12.

20. A vector comprising the nucleic acid construct of claim 13.

21. A vector comprising the nucleic acid construct of claim 14.

22. A vector comprising the nucleic acid construct of claim 15.

23. A vector comprising the nucleic acid construct of claim 16.

24. A cell transfected with the nucleic acid construct of claim 10.

25. A cell transformed by the nucleic acid construct of claim 10 .

26. A pharmaceutical composition comprising a therapeutically effective dose of the nucleic acid construct of claim 10 and a hormone.

27. A pharmaceutical composition comprising a therapeutically effective dose of the nucleic acid construct of claim 15 and progesterone.

28. A pharmaceutical composition comprising a therapeutically effective dose of the composition of matter of claim 1.

29. A pharmaceutical composition comprising a therapeutically effective dose of the composition of matter of claim 9.

30. A method of delivering into a cell a nucleic acid encoding a gene to be expressed in the cell comprising providing the composition of matter of claim 1 to an organism so that the hormone in the composition crosses the cell membrane by diffusion and transports the nucleic acid that is coupled to the hormone-hormone receptor complex across the membrane and into the cell.

31. The method of claim 30, wherein a nucleic acid encoding human Factor IX is delivered into the cell.

32. A method of treating a blood clotting disorder comprising administering a therapeutically effective amount of the composition of matter of claim 1 to an organism.

33. A method of treating hemophilia B, comprising administering a therapeutically effective amount of the composition of matter of claim 9 to an organism.

Figure 1

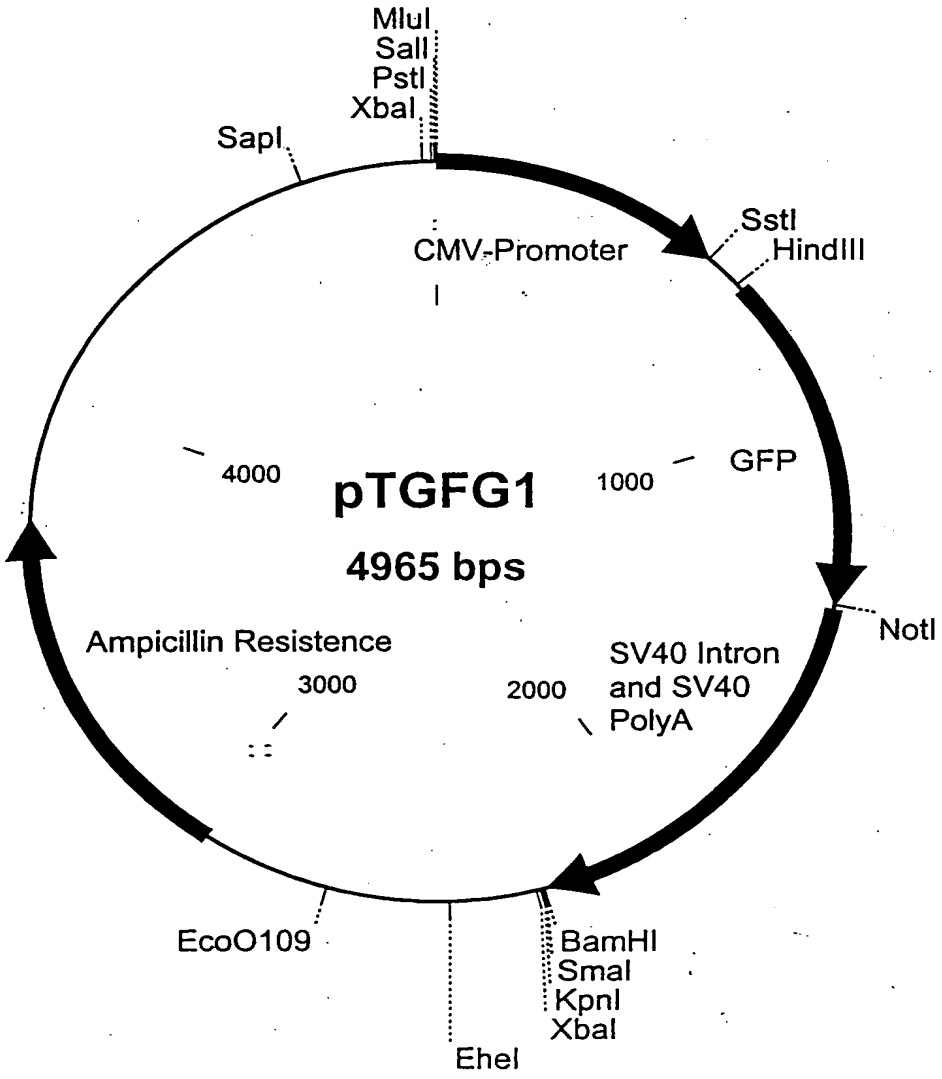

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
GFP
1000
4000
pTGFG1
4965 bps
NotI
Ampicillin Resistence
3000
2000
SV40 Intron
and SV40
PolyA
EcoO109
EheI
BamHI
SmaI
KpnI
XbaI

Figure 2

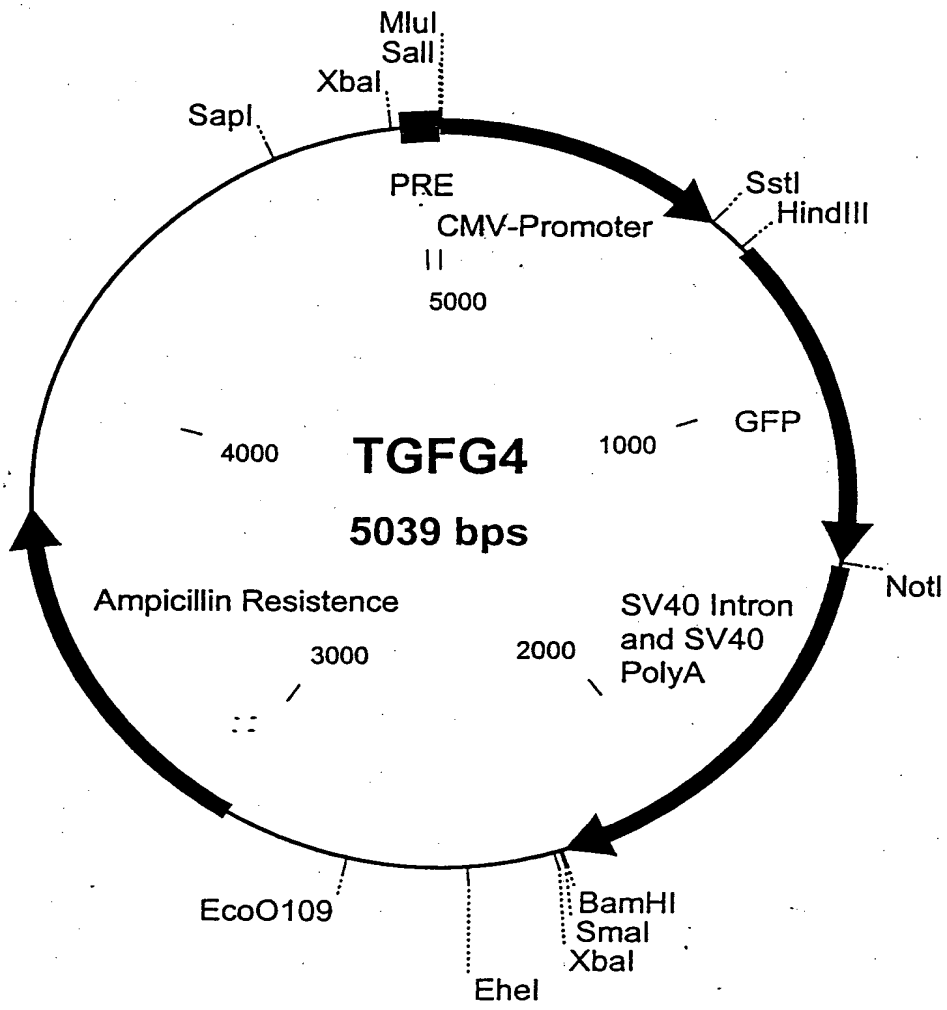

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
5000
SstI
HindIII
GFP
1000
TGFG4
5039 bps
4000
NotI
Ampicillin Resistence
3000
SV40 Intron
and SV40
PolyA
2000
EcoO109
EheI
BamHI
SmaI
XbaI

Figure 3

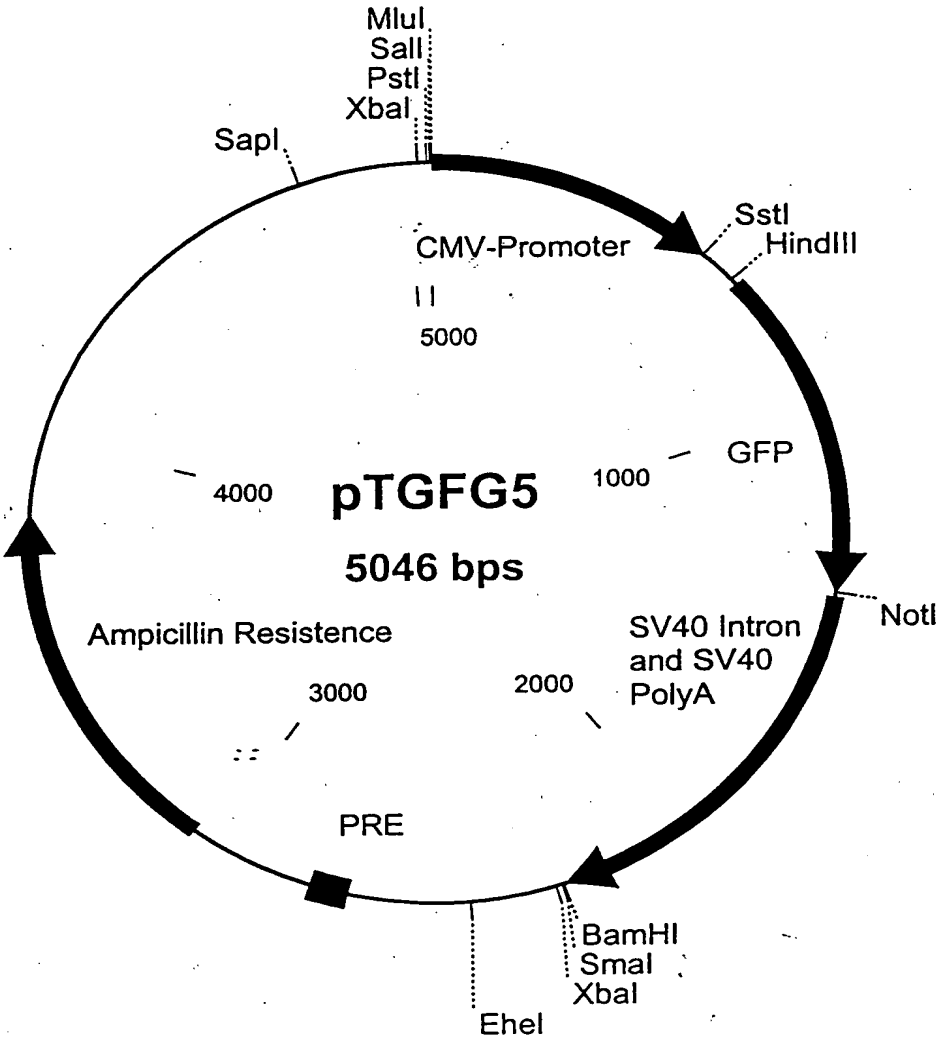

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
1000
GFP
4000
pTGFG5
5046 bps
NotI
Ampicillin Resistence
SV40 Intron
and SV40
PolyA
3000
2000
PRE
BamHI
SmaI
XbaI
EheI

Figure 4

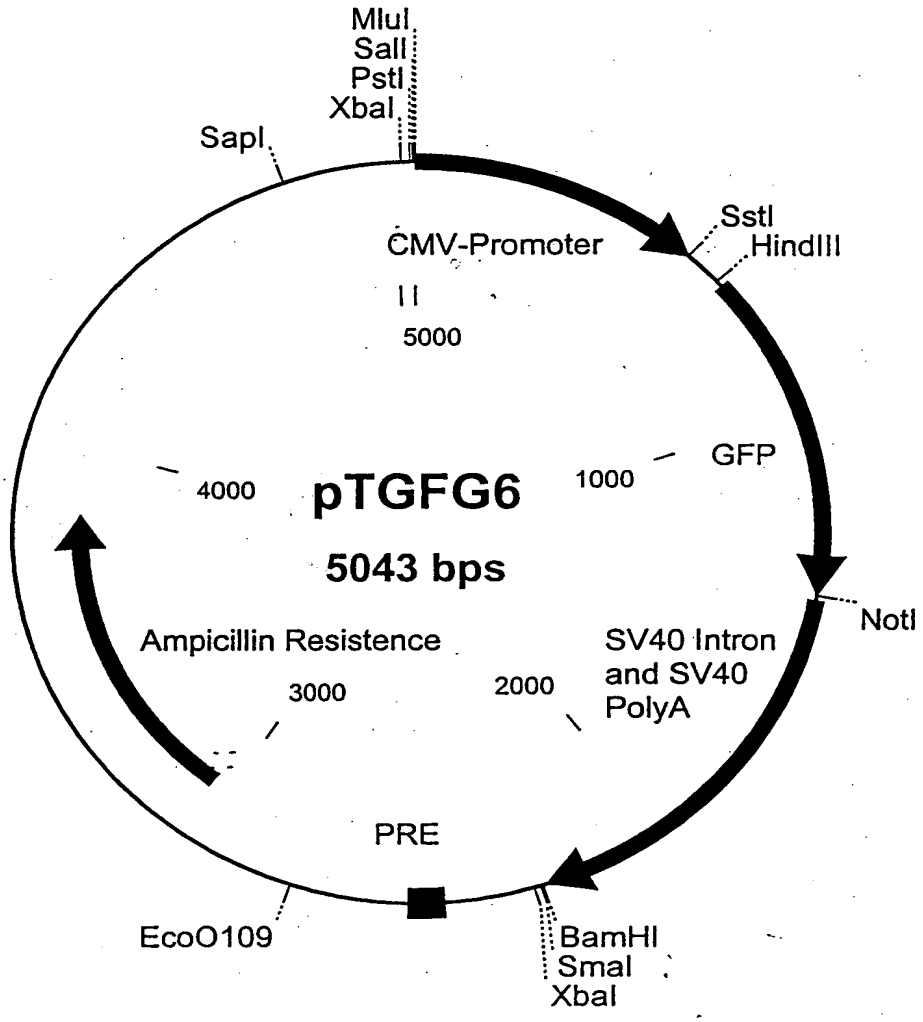

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
4000
pTGFG6
5043 bps
NotI
Ampicillin Resistence
3000
2000
SV40 Intron
and SV40
PolyA
PRE
EcoO109
BamHI
SmaI
XbaI

Figure 5

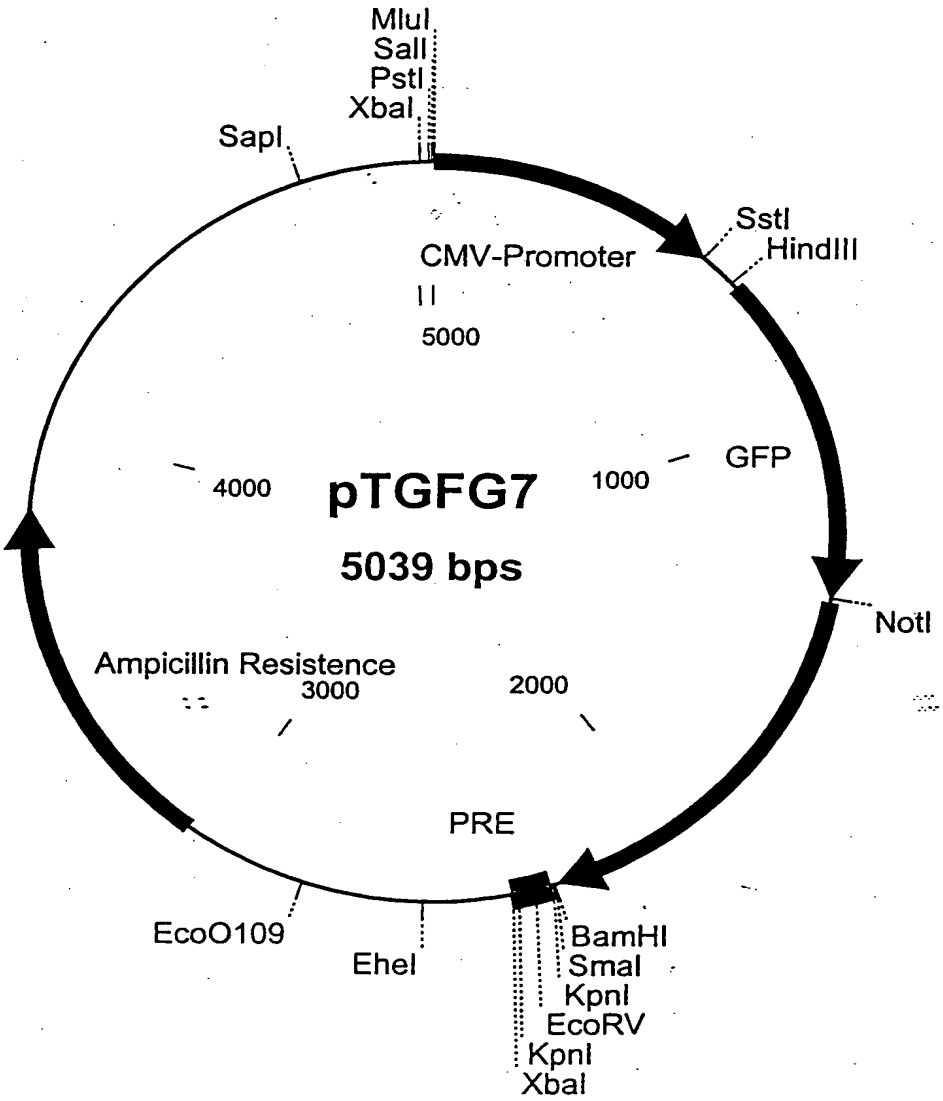

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
pTGFG7
5039 bps
4000
NotI
Ampicillin Resistence
3000
2000
PRE
EcoO109
EheI
BamHI
SmaI
KpnI
EcoRV
KpnI
XbaI

Figure 6

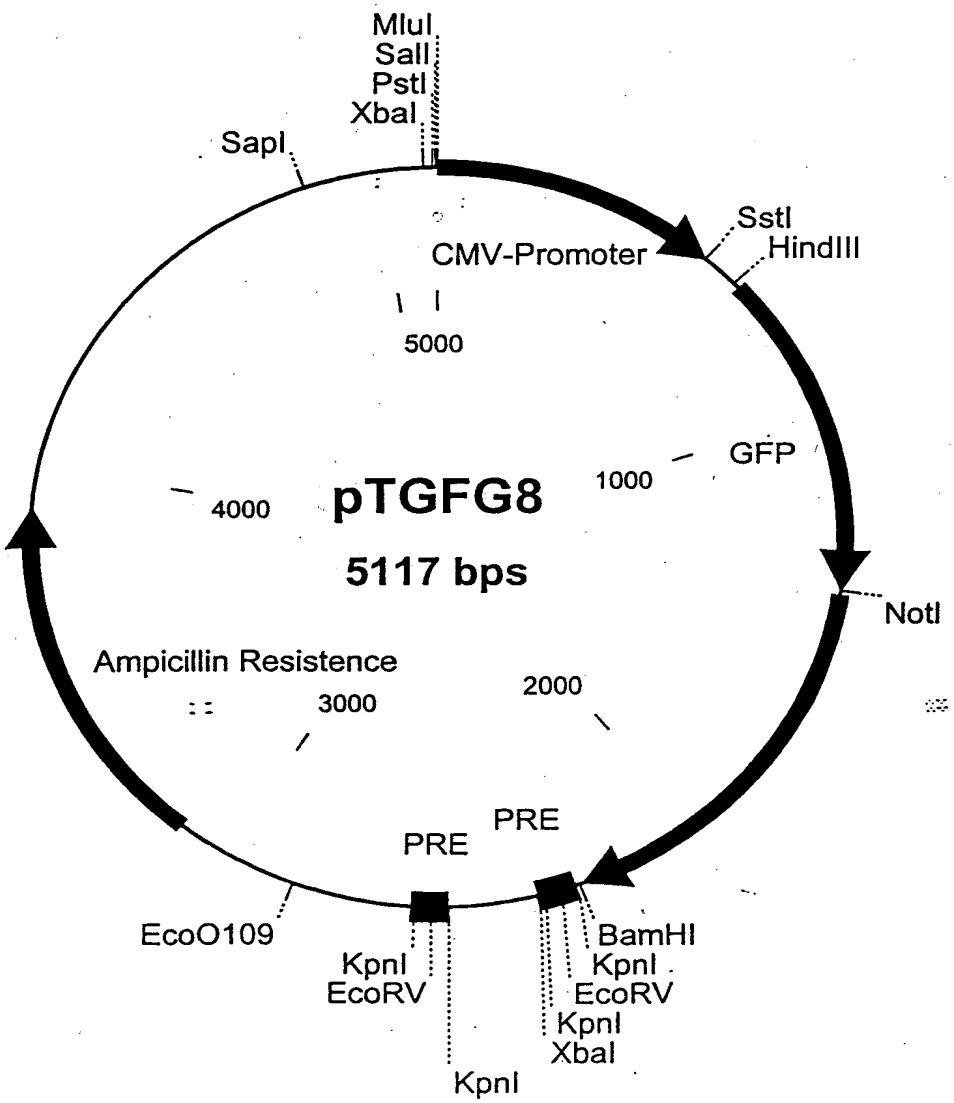

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
4000
pTGFG8
5117 bps
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
EcoO109
KpnI
EcoRV
KpnI
BamHI
KpnI
EcoRV
KpnI
XbaI

Figure 7

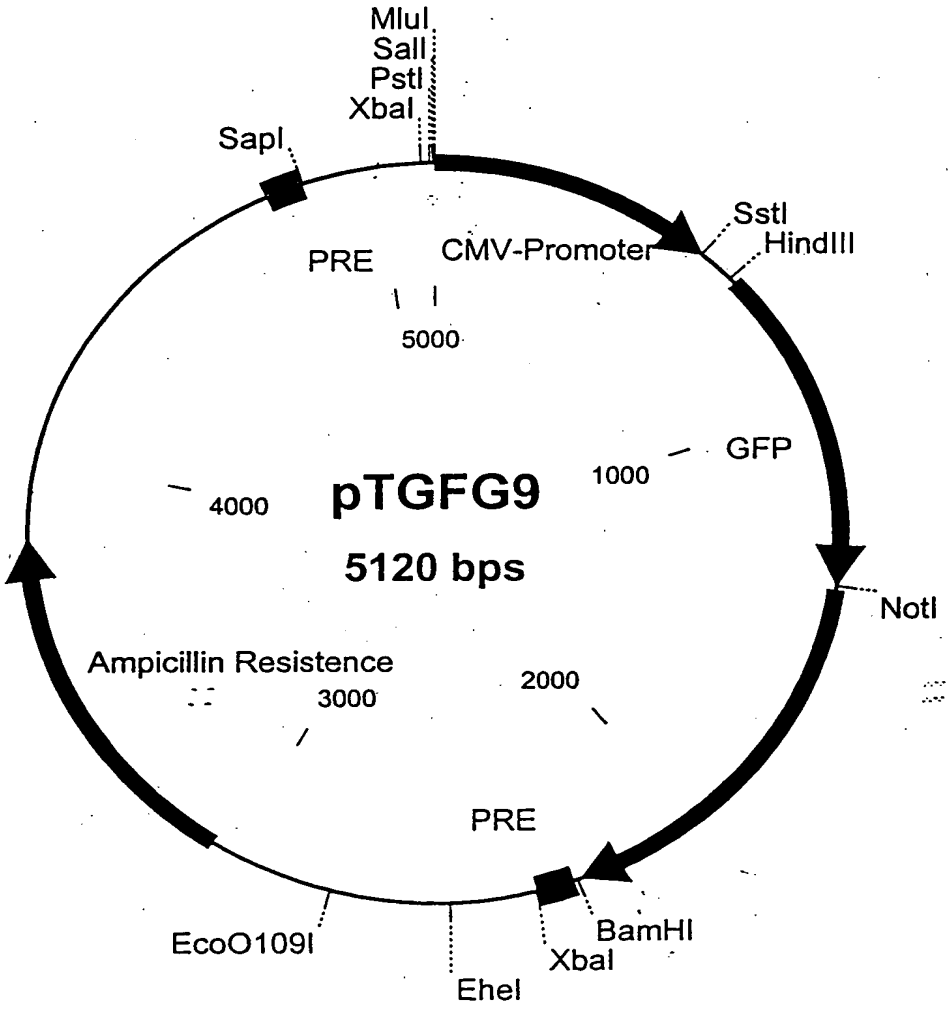

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
4000
pTGFG9
5120 bps
NotI
Ampicillin Resistence
3000
2000
PRE
BamHI
XbaI
EcoO109I
EheI

Figure 8

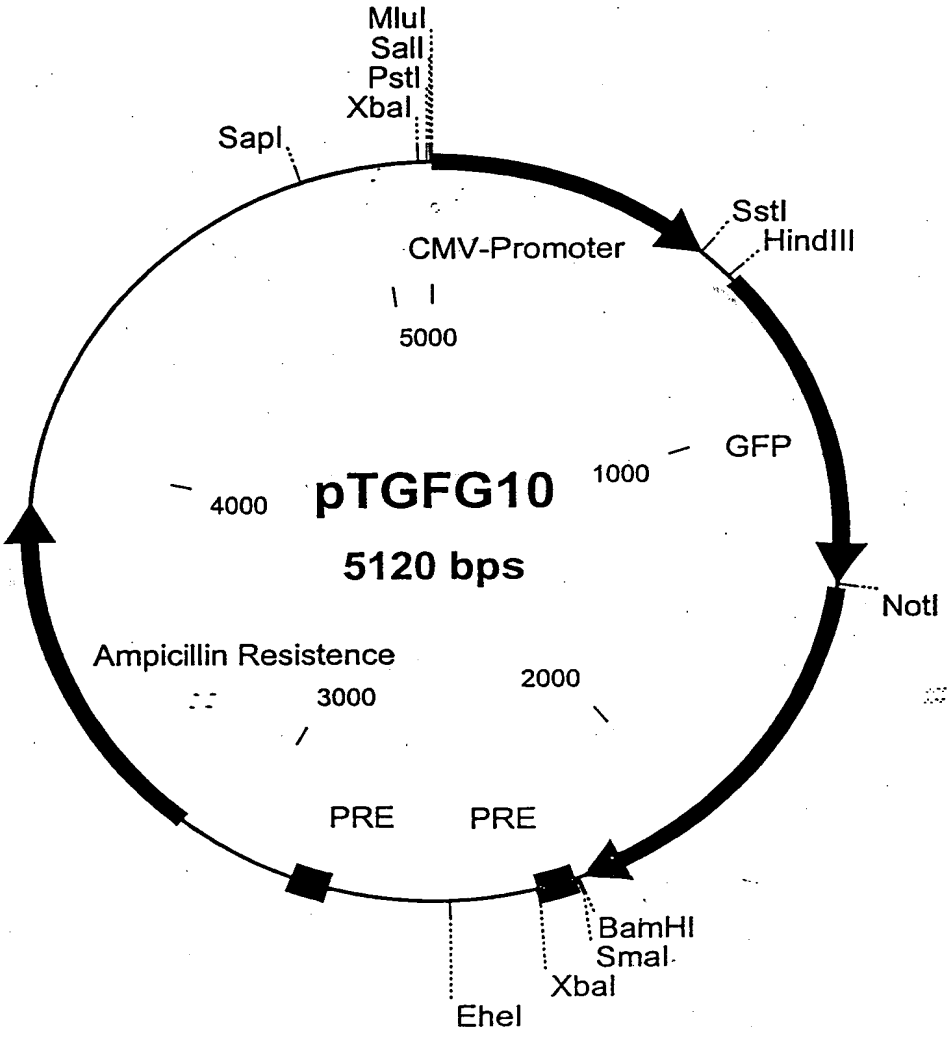

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
4000
pTGFG10
5120 bps
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
BamHI
SmaI
XbaI
EheI

Figure 9

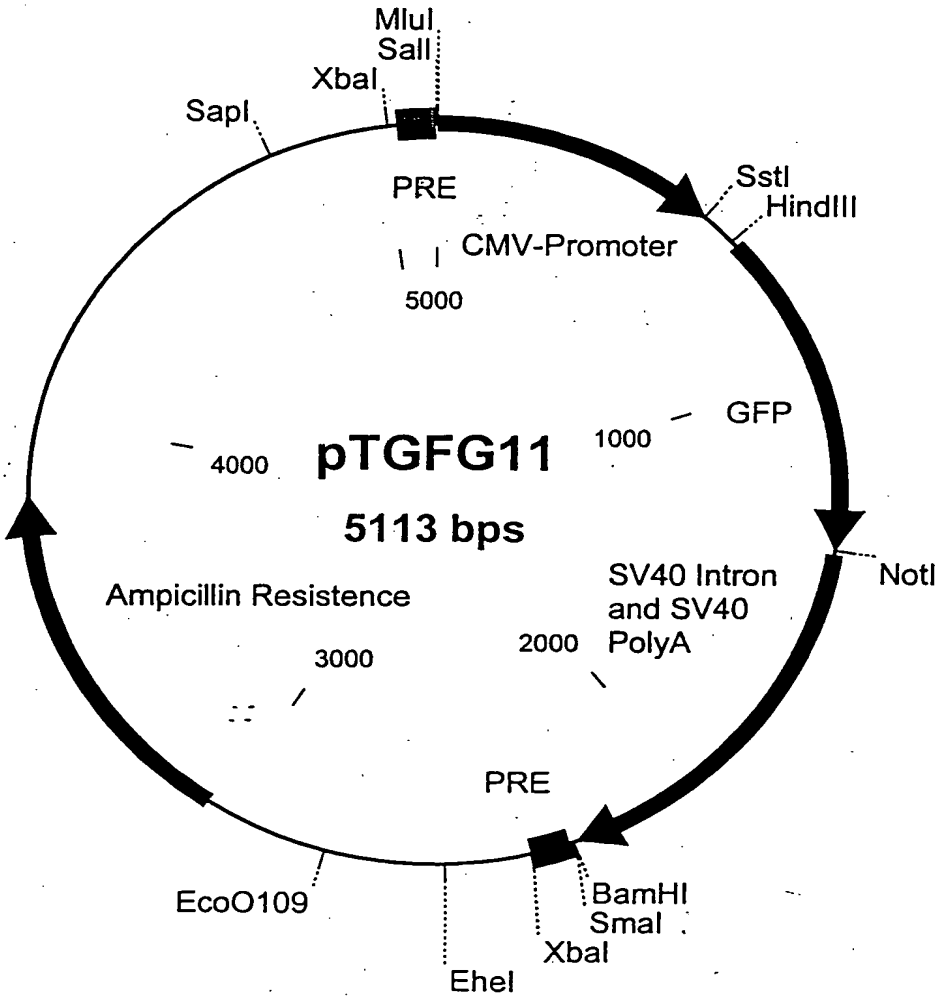

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
CMV-Promoter
5000
GFP
1000
4000
pTGFG11
5113 bps
NotI
SV40 Intron
and SV40
PolyA
2000
Ampicillin Resistence
3000
PRE
BamHI
SmaI
XbaI
EcoO109
EheI

Figure 10

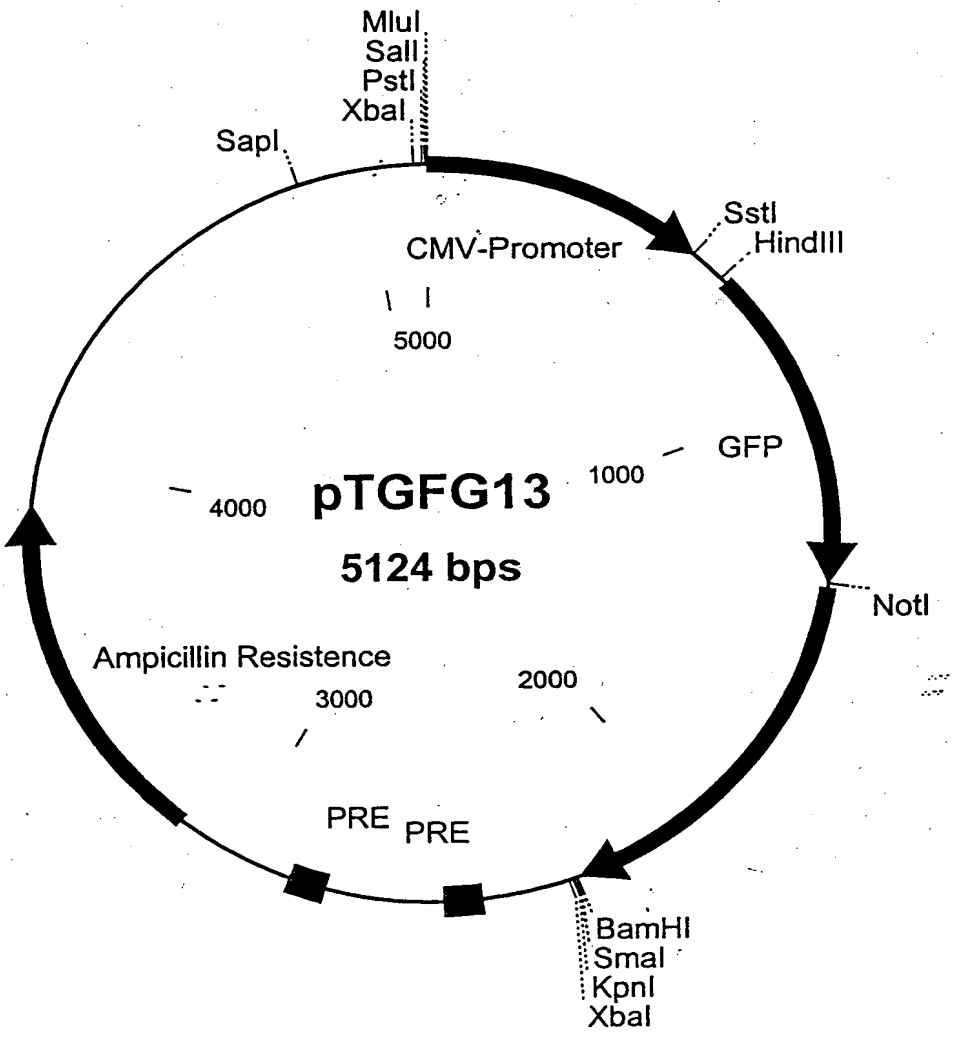

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
pTGFG13
5124 bps
4000
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
BamHI
SmaI
KpnI
XbaI

Figure 11

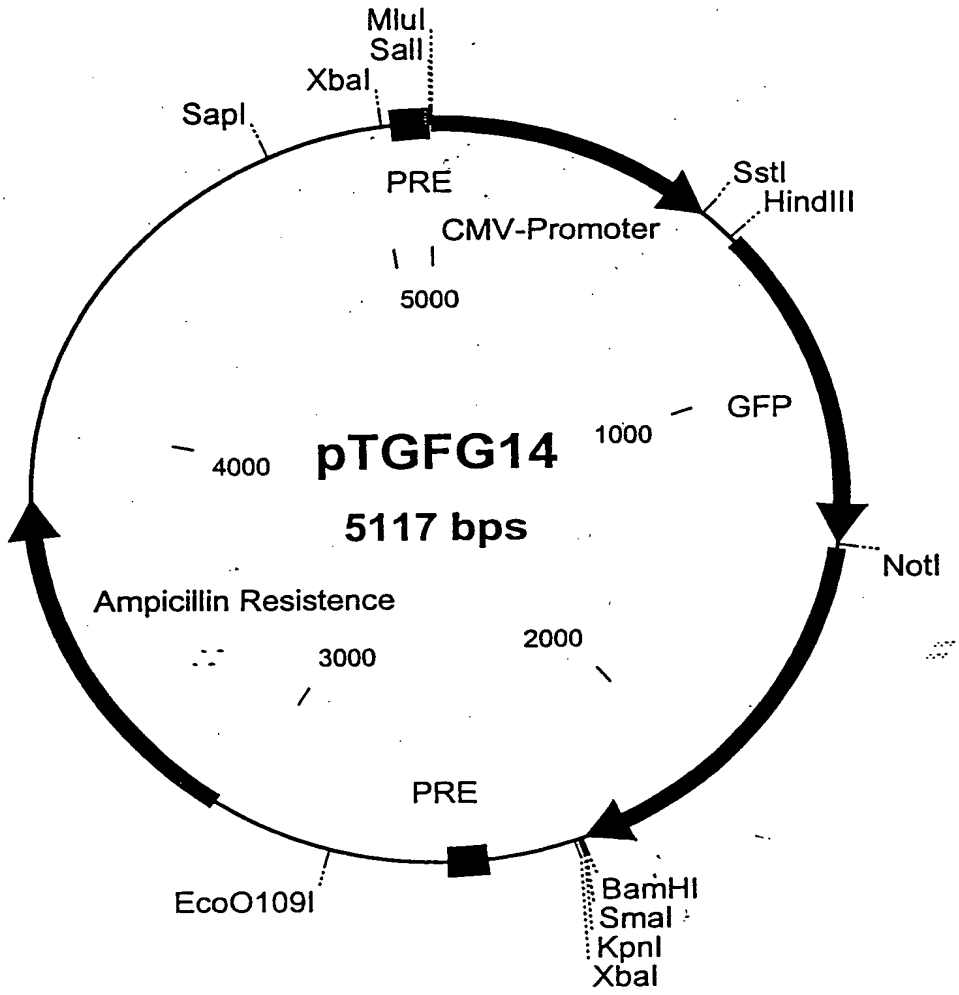

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
CMV-Promoter
5000
GFP
1000
4000
pTGFG14
5117 bps
NotI
Ampicillin Resistence
3000
2000
PRE
EcoO109I
BamHI
SmaI
KpnI
XbaI

Figure 12

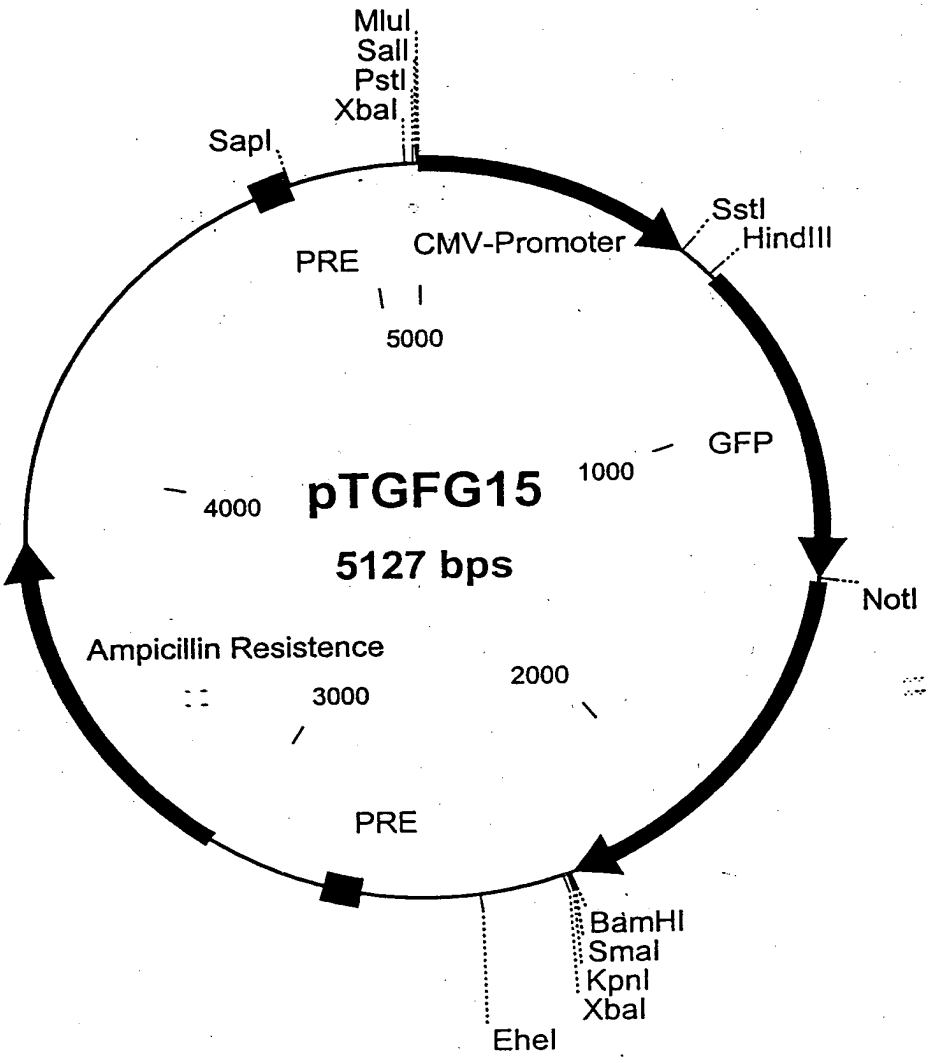

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
5000
GFP
1000
pTGFG15
5127 bps
4000
NotI
Ampicillin Resistence
3000
2000
PRE
BamHI
SmaI
KpnI
XbaI
EheI

Figure 13

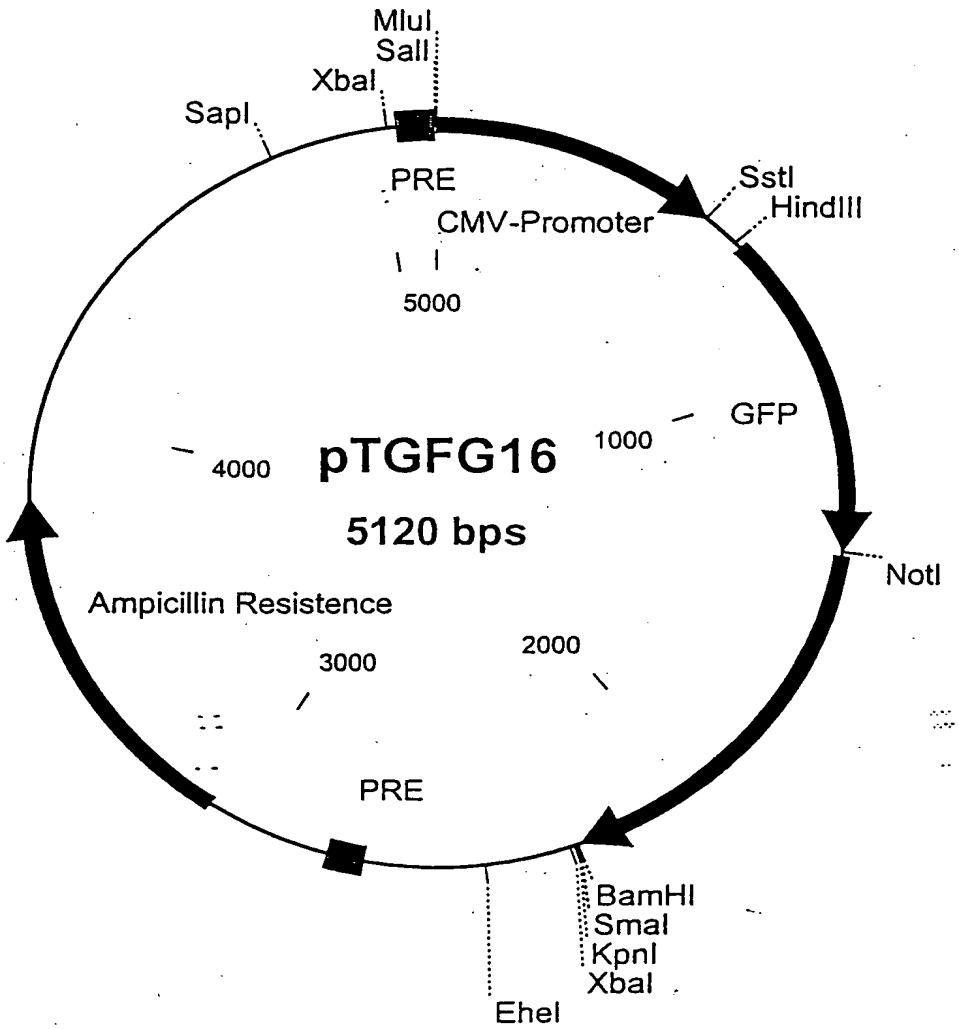

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
SstI
HindIII
5000
GFP
1000
4000
pTGFG16
5120 bps
NotI
Ampicillin Resistence
3000
2000
PRE
BamHI
SmaI
KpnI
XbaI
EheI

Figure 14

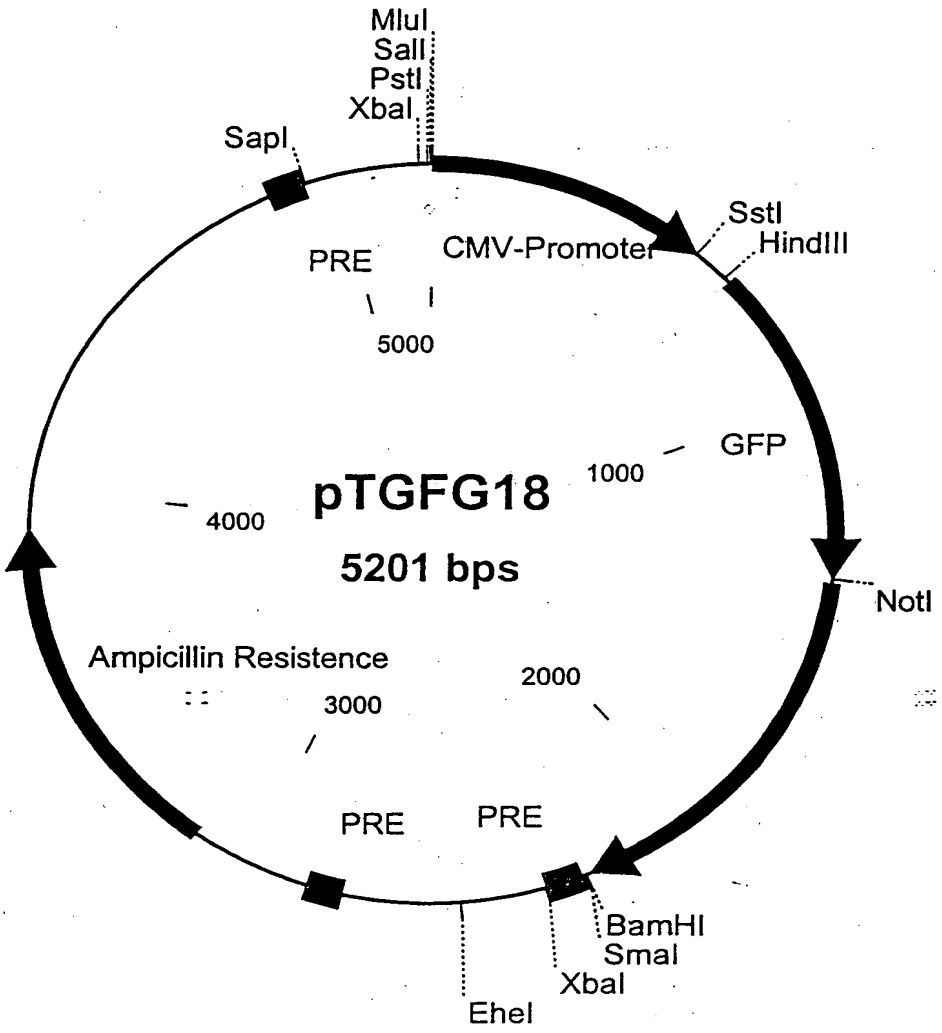

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
5000
GFP
1000
pTGFG18
5201 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
BamHI
SmaI
XbaI
EheI

Figure 15

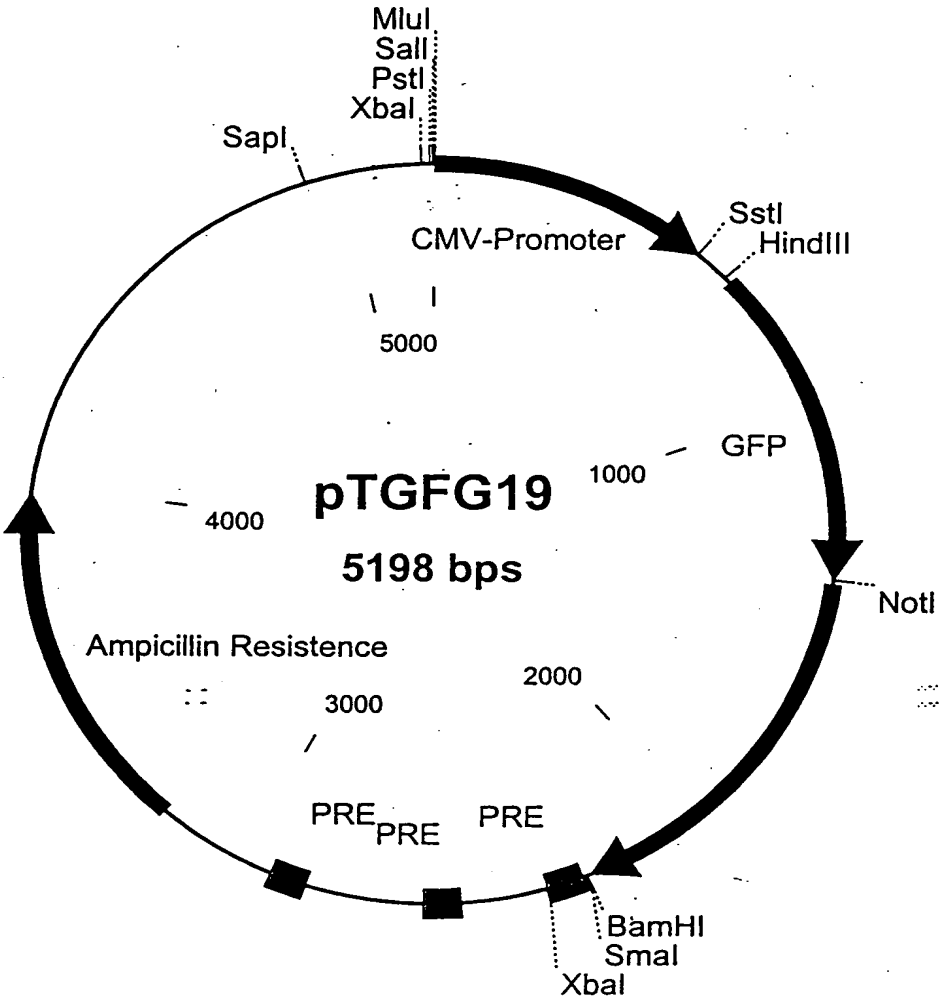

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
5000
GFP
1000
pTGFG19
5198 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
PRE
BamHI
SmaI
XbaI

Figure 16

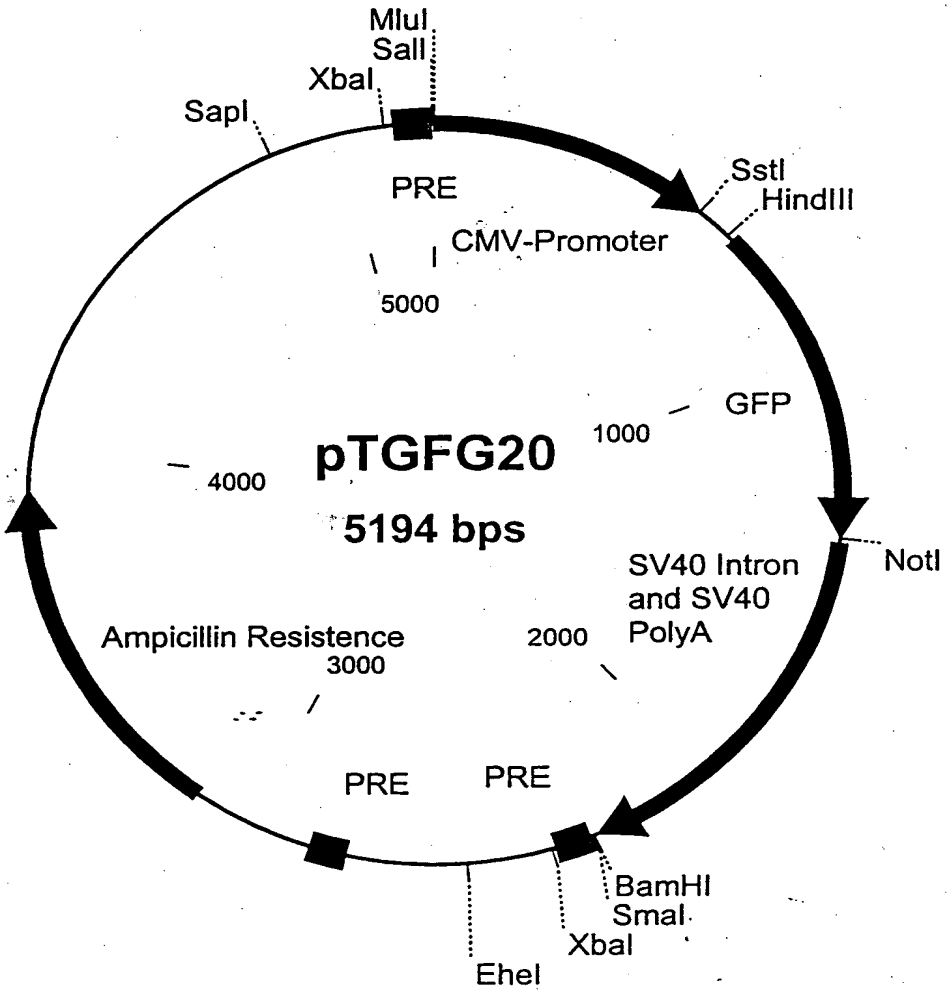

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
CMV-Promoter
5000
GFP
1000
pTGFG20
5194 bps
4000
SV40 Intron
and SV40
PolyA
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
BamHI
SmaI
XbaI
EheI

Figure 17

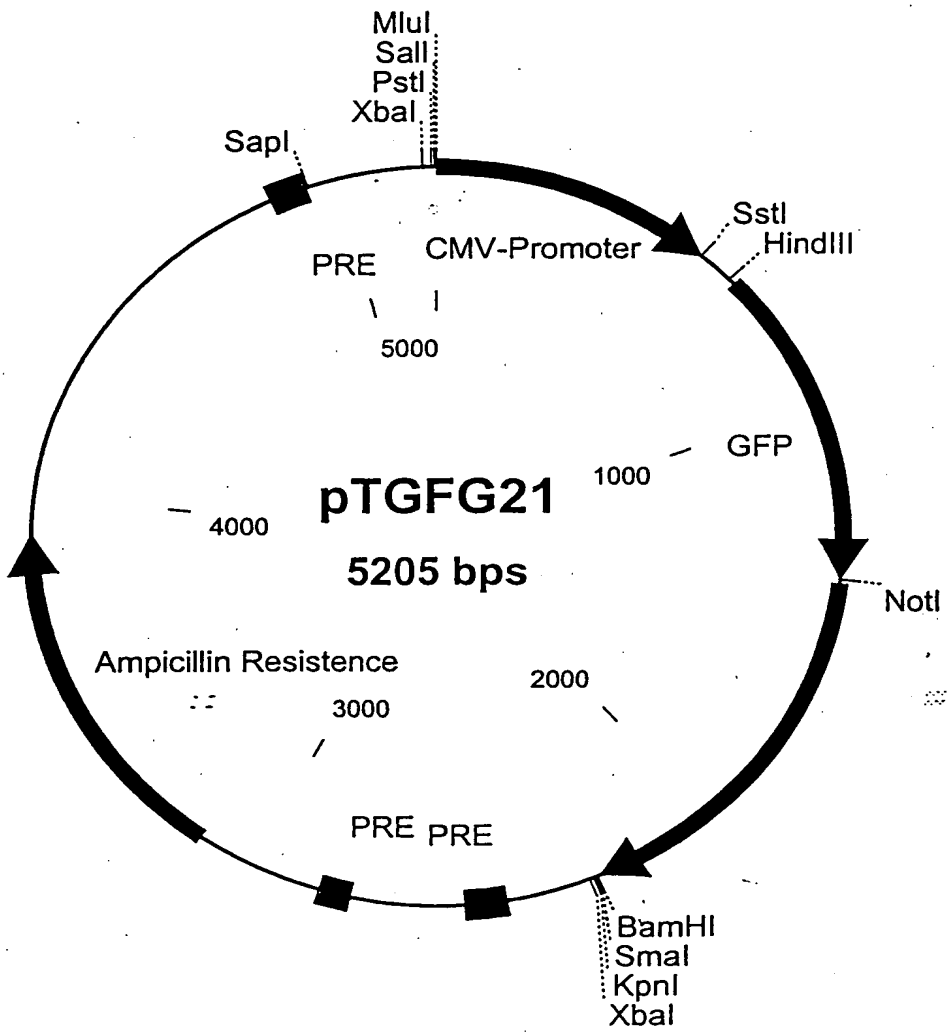

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
5000
GFP
1000
pTGFG21
5205 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
BamHI
SmaI
KpnI
XbaI

Figure 18

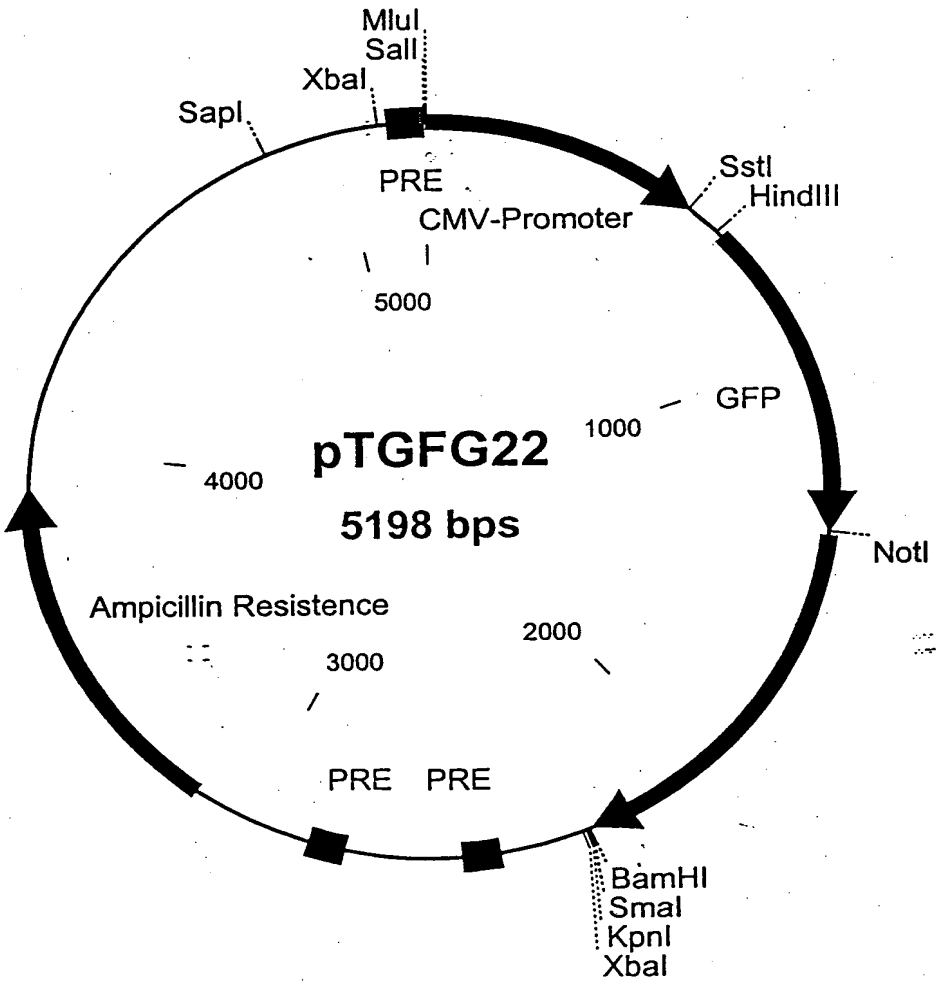

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
5000
SstI
HindIII
GFP
1000
pTGFG22
5198 bps
4000
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
BamHI
SmaI
KpnI
XbaI

Figure 19

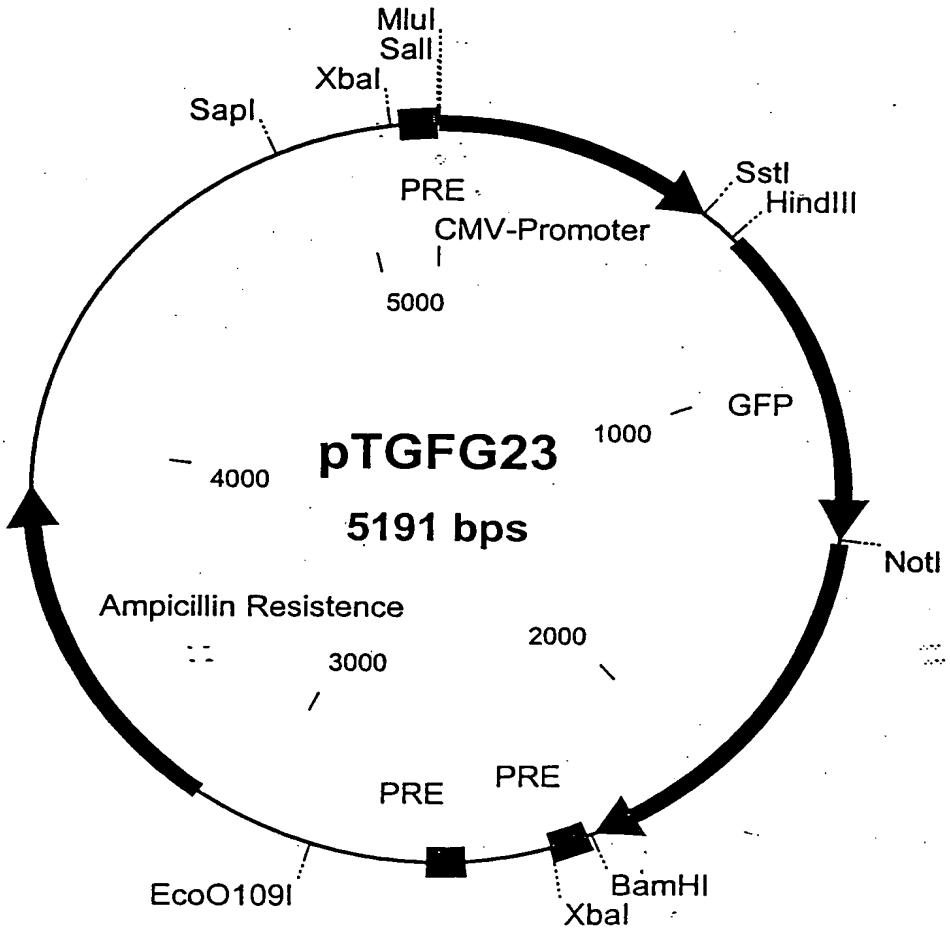

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
SstI
HindIII
5000
GFP
1000
pTGFG23
5191 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
EcoO109I
BamHI
XbaI

Figure 20

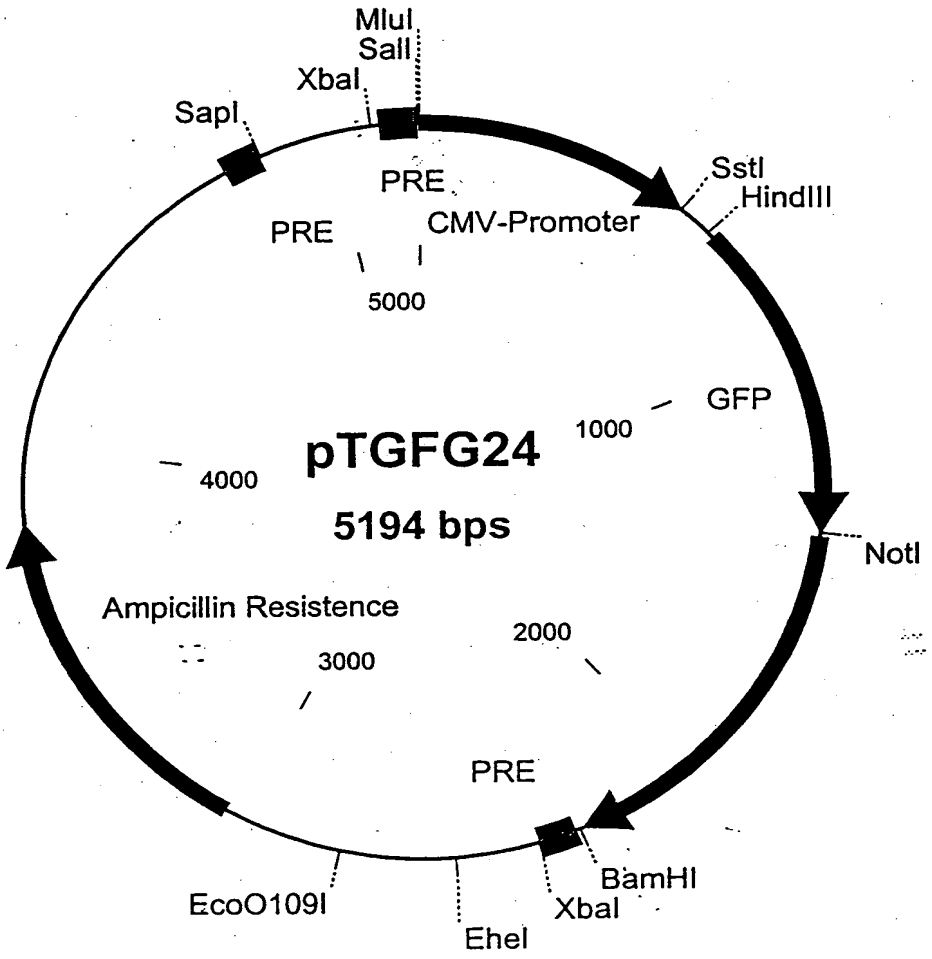

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
pTGFG24
4000
5194 bps
NotI
Ampicillin Resistence
2000
3000
PRE
BamHI
XbaI
EcoO109I
EheI

Figure 21

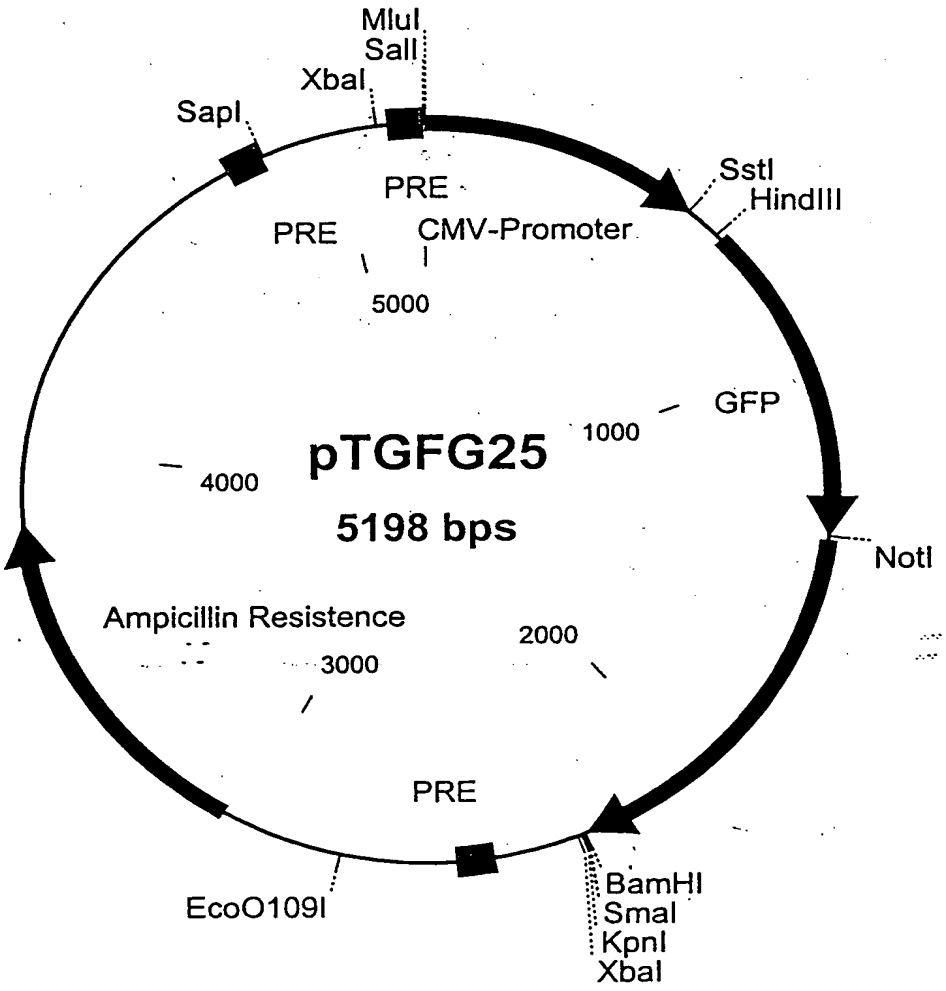

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
pTGFG25
4000
5198 bps
NotI
Ampicillin Resistence
2000
3000
PRE
BamHI
SmaI
KpnI
XbaI
EcoO109I

Figure 22

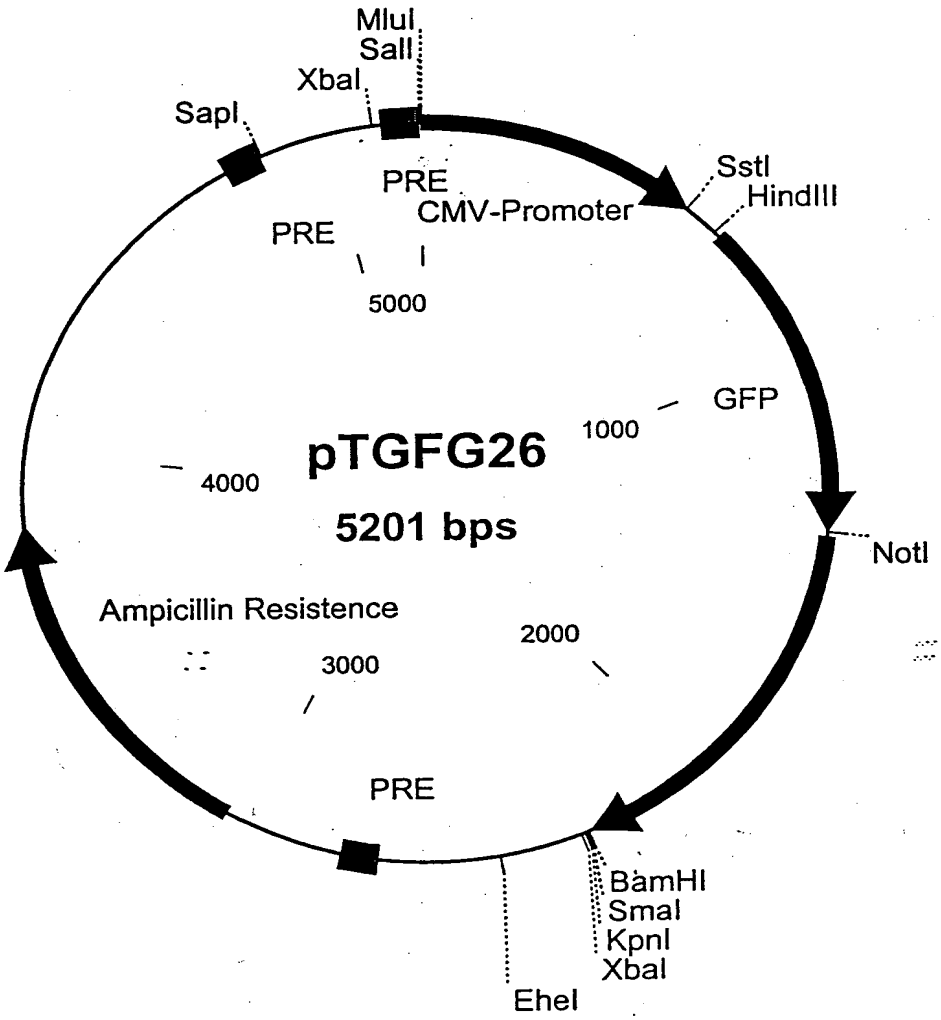

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
SstI
HindIII
PRE
5000
GFP
1000
pTGFG26
5201 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
BamHI
SmaI
KpnI
XbaI
EheI

Figure 23

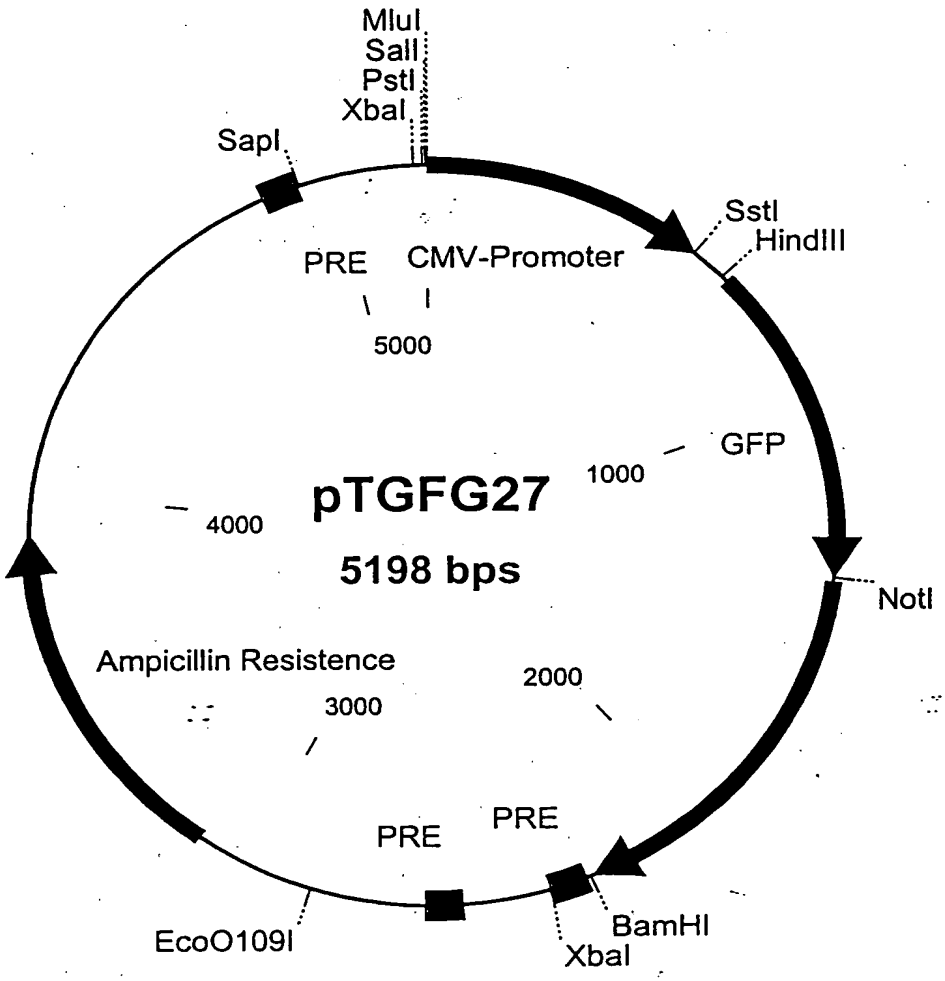

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
pTGFG27
5198 bps
4000
NotI
Ampicillin Resistence
3000
2000
PRE
PRE
EcoO109I
BamHI
XbaI

Figure 24

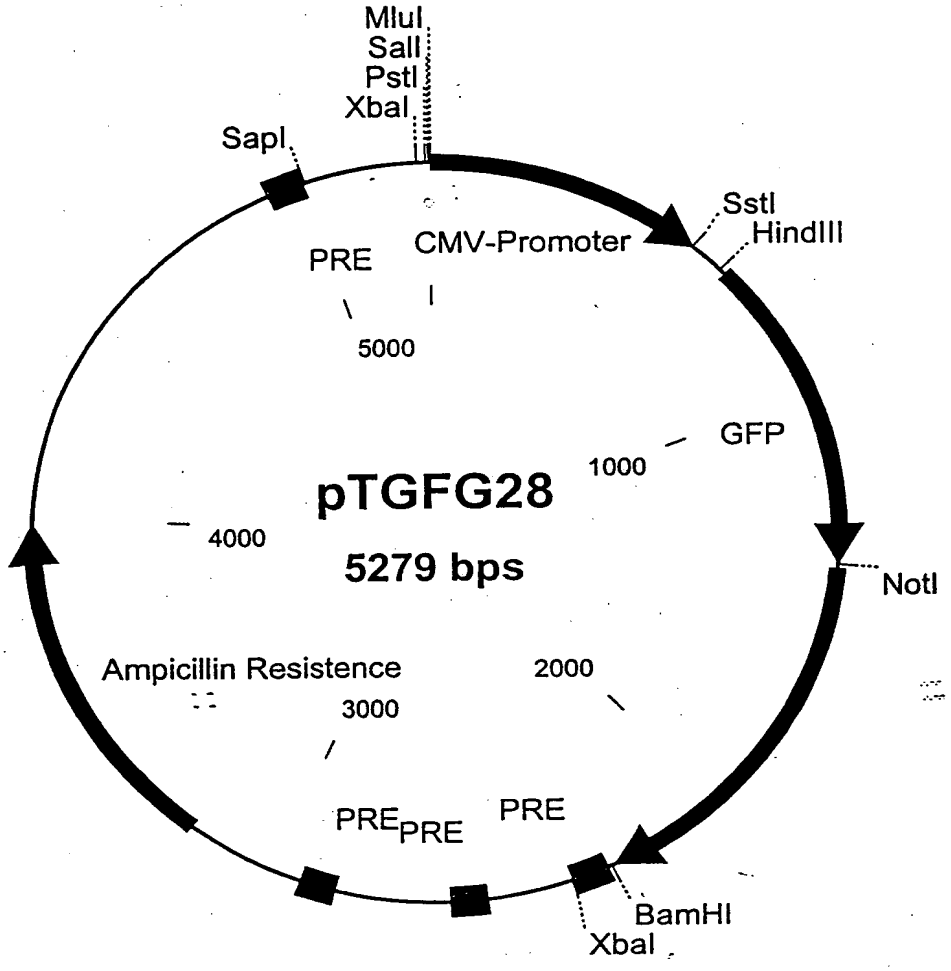

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
5000
GFP
1000
pTGFG28
5279 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
PRE
BamHI
XbaI

Figure 25

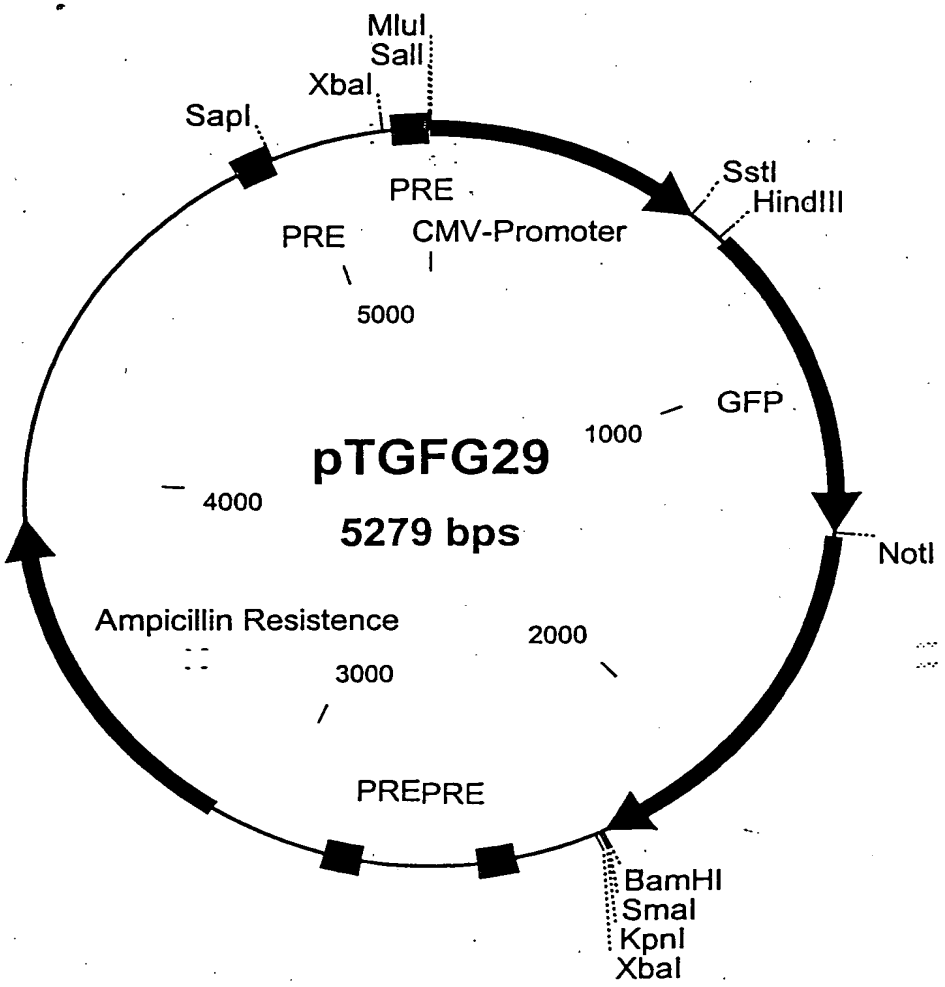

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
pTGFG29
5279 bps
4000
NotI
Ampicillin Resistence
2000
3000
PREPRE
BamHI
SmaI
KpnI
XbaI

Figure 26

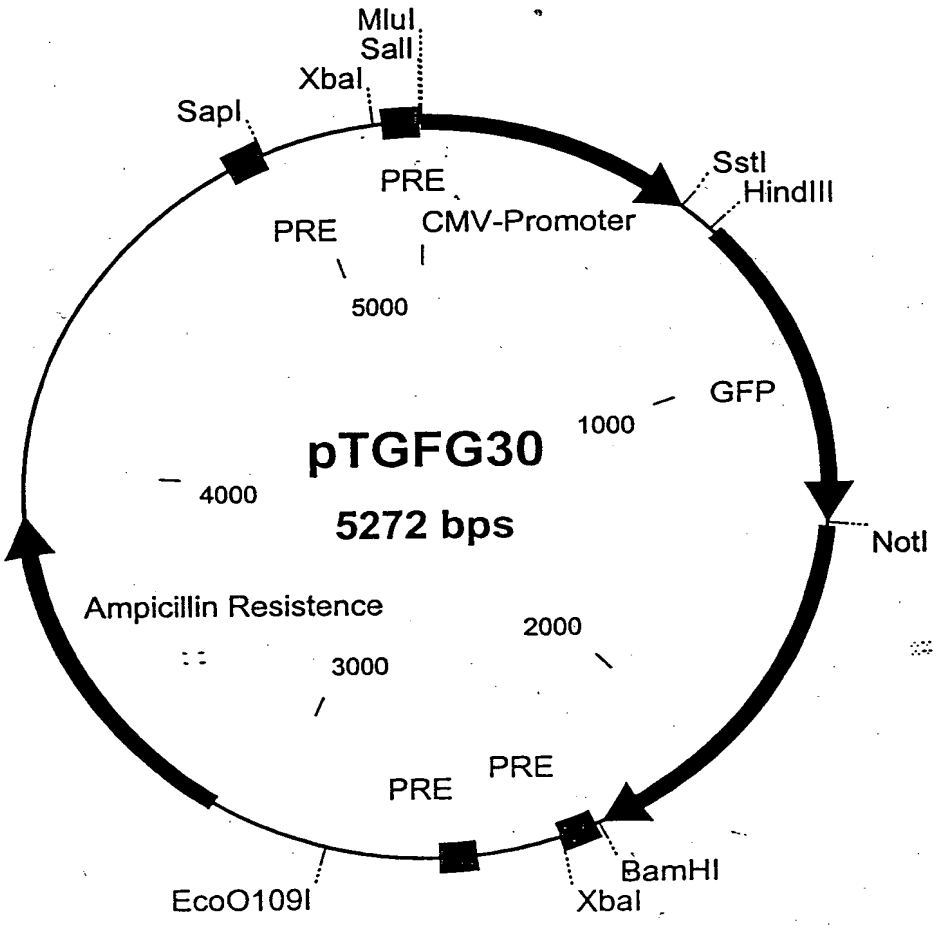

MluI
SalI
XbaI
SapI
PRE
SstI
HindIII
PRE
CMV-Promoter
5000
GFP
1000
pTGFG30
5272 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
BamHI
XbaI
EcoO109I

Figure 27

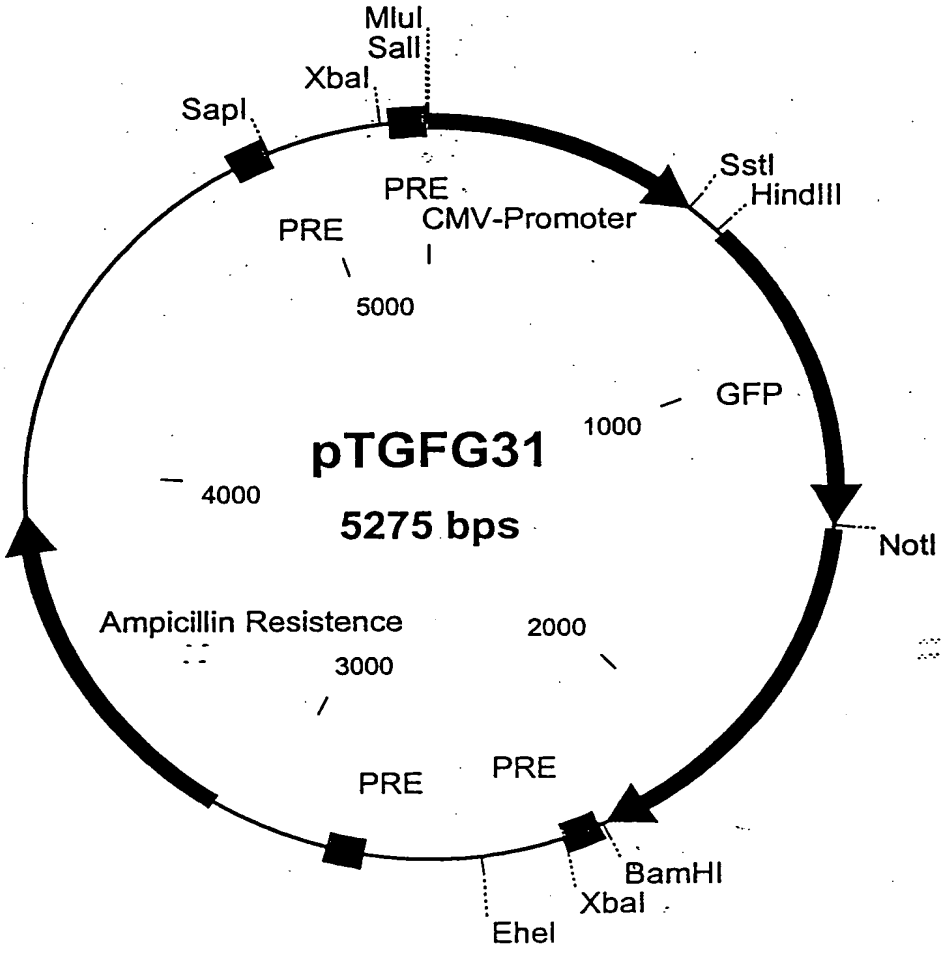

MluI
SalI
XbaI
SapI
SstI
HindIII
PRE
CMV-Promoter
PRE
5000
GFP
1000
pTGFG31
5275 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
BamHI
XbaI
EheI

Figure 28

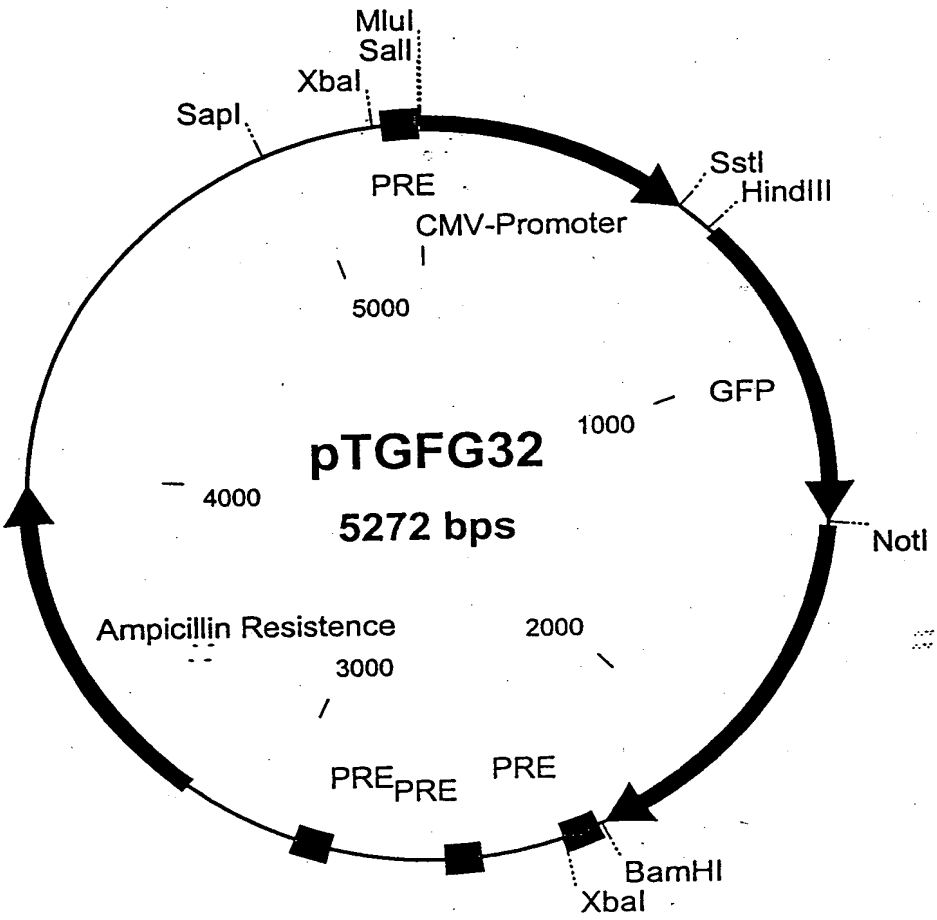

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
SstI
HindIII
5000
GFP
1000
pTGFG32
5272 bps
4000
NotI
Ampicillin Resistence
2000
3000
PRE
PRE
PRE
BamHI
XbaI

Figure 29

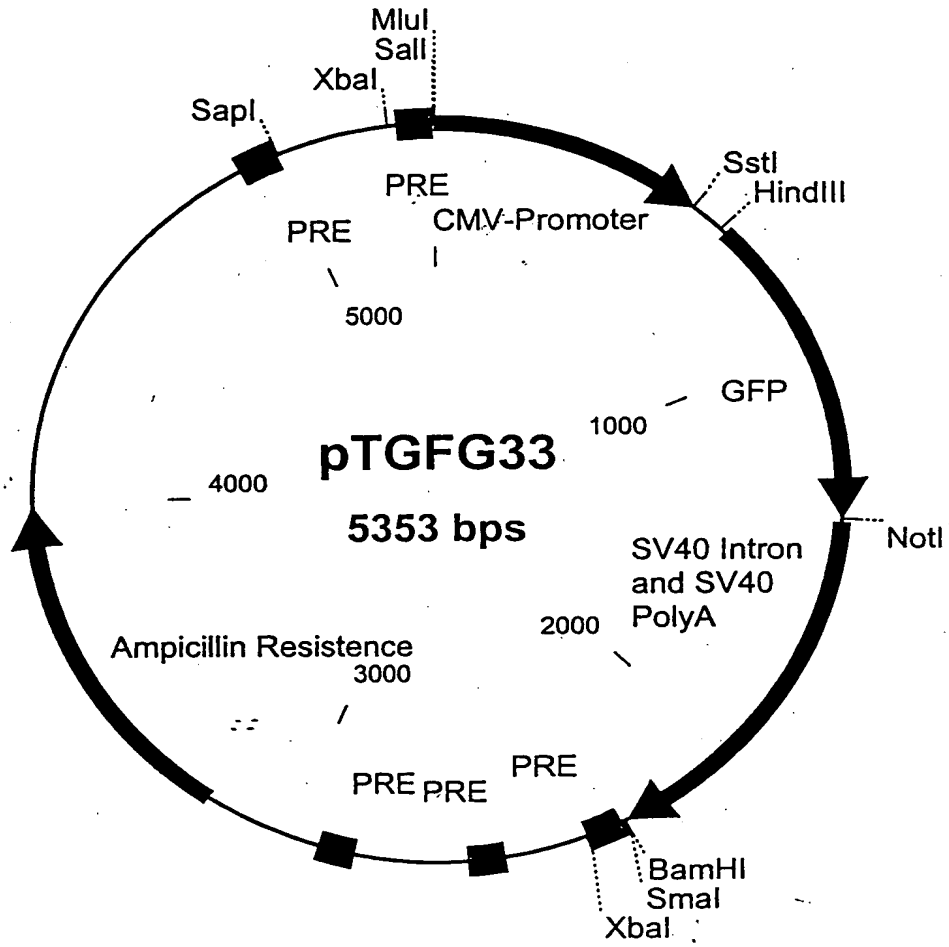

MluI
SalI
XbaI
SapI
PRE
PRE
CMV-Promoter
5000
SstI
HindIII
GFP
1000
pTGFG33
5353 bps
4000
SV40 Intron
and SV40
PolyA
NotI
2000
Ampicillin Resistence
3000
PRE
PRE
PRE
BamHI
SmaI
XbaI

Figure 30

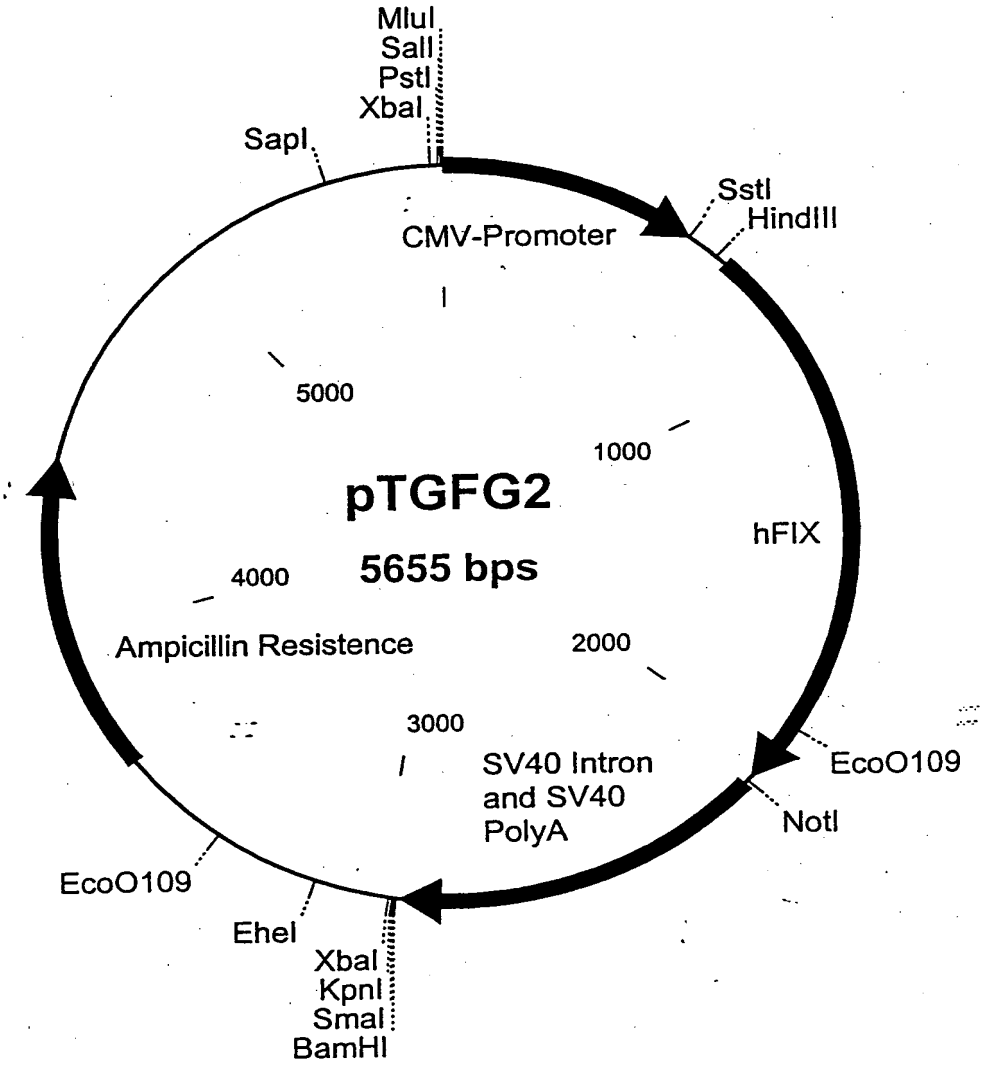

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
1
5000
1000
pTGFG2
5655 bps
hFIX
4000
Ampicillin Resistence
2000
3000
SV40 Intron
and SV40
PolyA
EcoO109
NotI
EcoO109
EheI
XbaI
KpnI
SmaI
BamHI

Figure 31

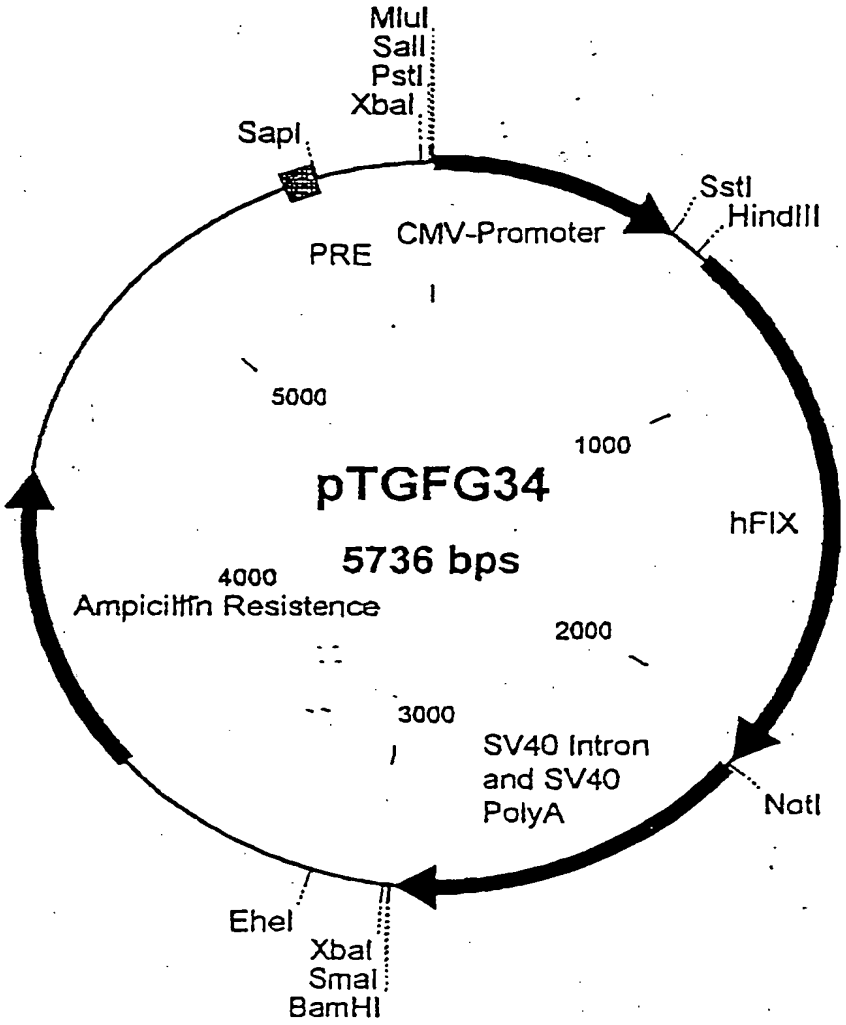

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
5000
1000
pTGFG34
5736 bps
hFIX
4000
Ampicillin Resistence
2000
3000
SV40 Intron
and SV40
PolyA
NotI
EheI
XbaI
SmaI
BamHI

Figure 32

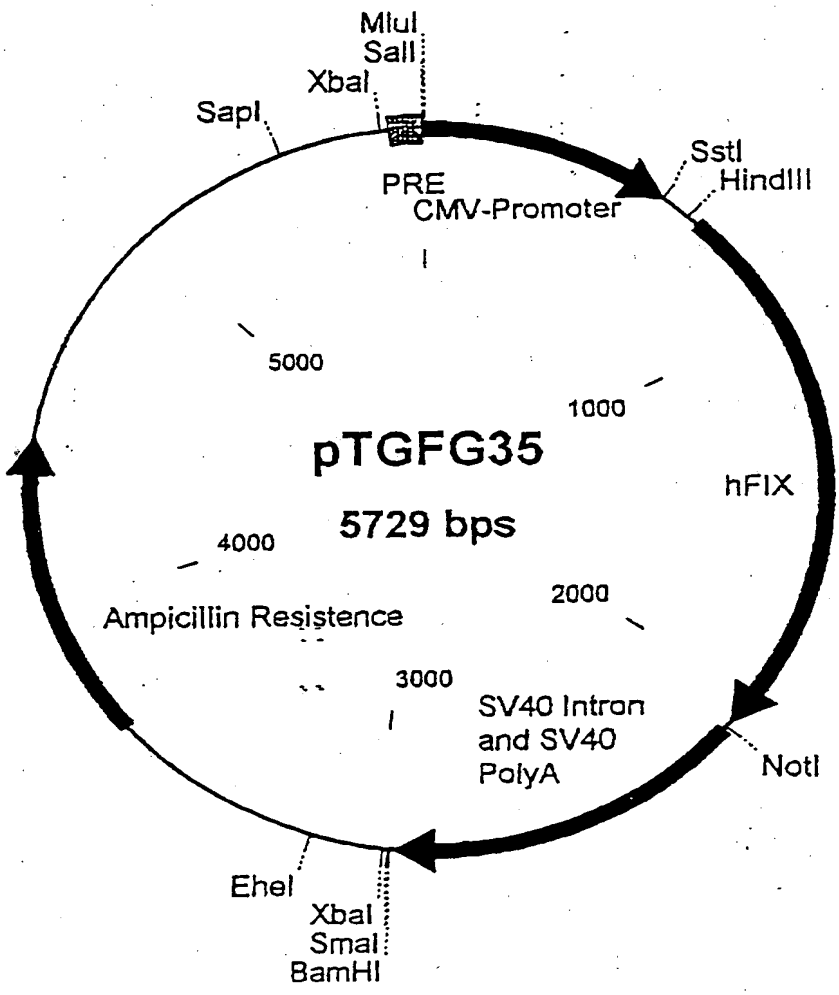

MluI
SalI
XbaI
SapI
SstI
HindIII
PRE
CMV-Promoter
5000
1000
pTGFG35
5729 bps
hFIX
4000
2000
Ampicillin Resistence
3000
SV40 Intron
and SV40
PolyA
NotI
EheI
XbaI
SmaI
BamHI

Figure 33

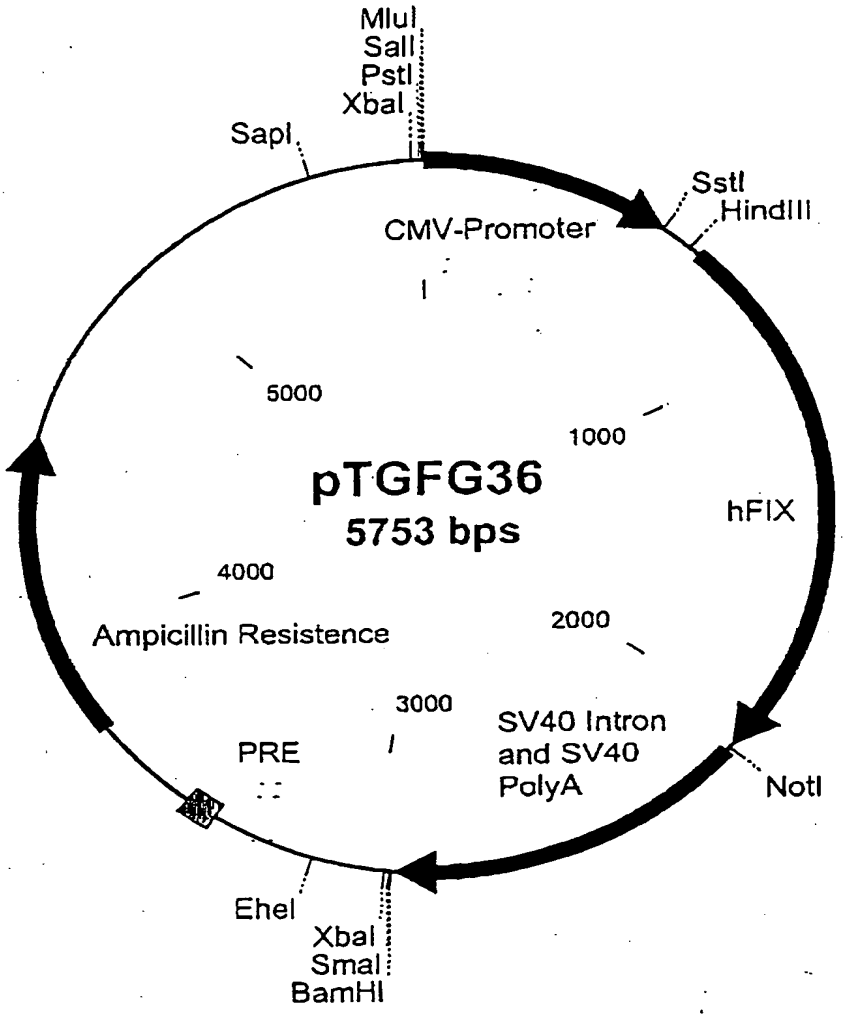

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
1000
hFIX
2000
NotI
SV40 Intron
and SV40
PolyA
3000
PRE
EheI
XbaI
SmaI
BamHI
4000
Ampicillin Resistence
5000
pTGFG36
5753 bps

Figure 34

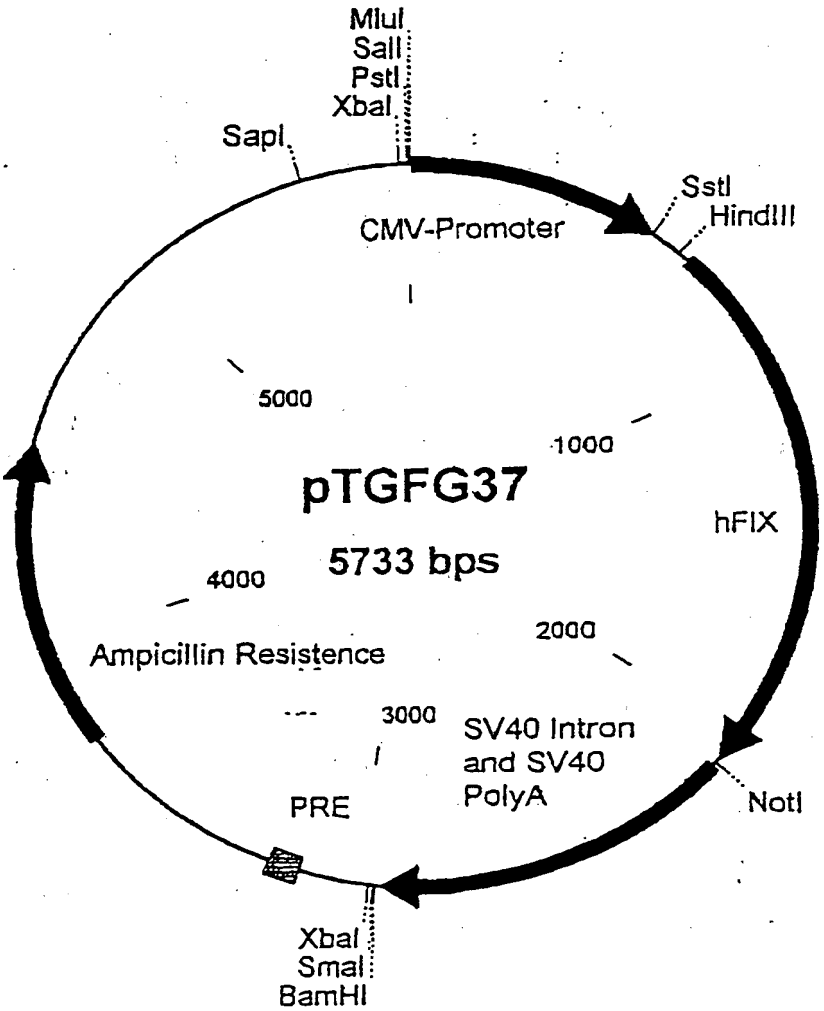

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
1000
hFIX
2000
NotI
SV40 Intron
and SV40
PolyA
3000
PRE
XbaI
SmaI
BamHI
4000
Ampicillin Resistence
5000
pTGFG37
5733 bps

Figure 35

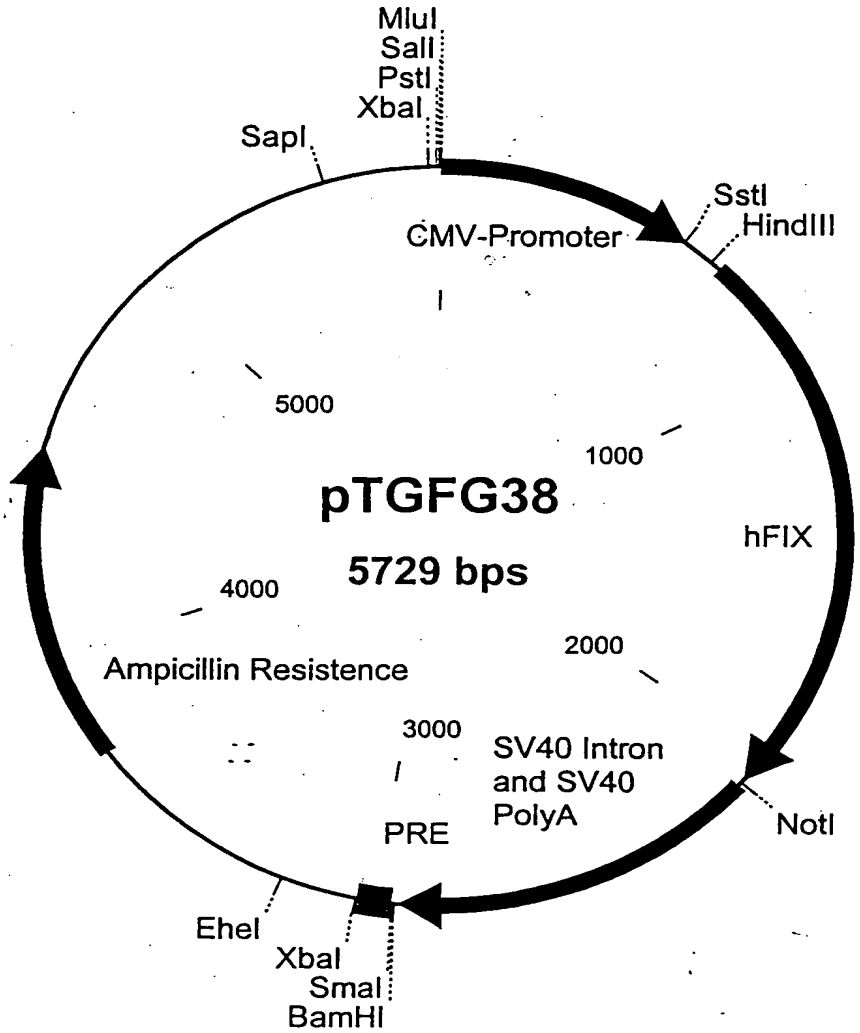

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
CMV-Promoter
1
5000
1000
pTGFG38
5729 bps
hFIX
4000
2000
Ampicillin Resistence
3000
SV40 Intron
and SV40
PolyA
PRE
NotI
EheI
XbaI
SmaI
BamHI

Figure 36

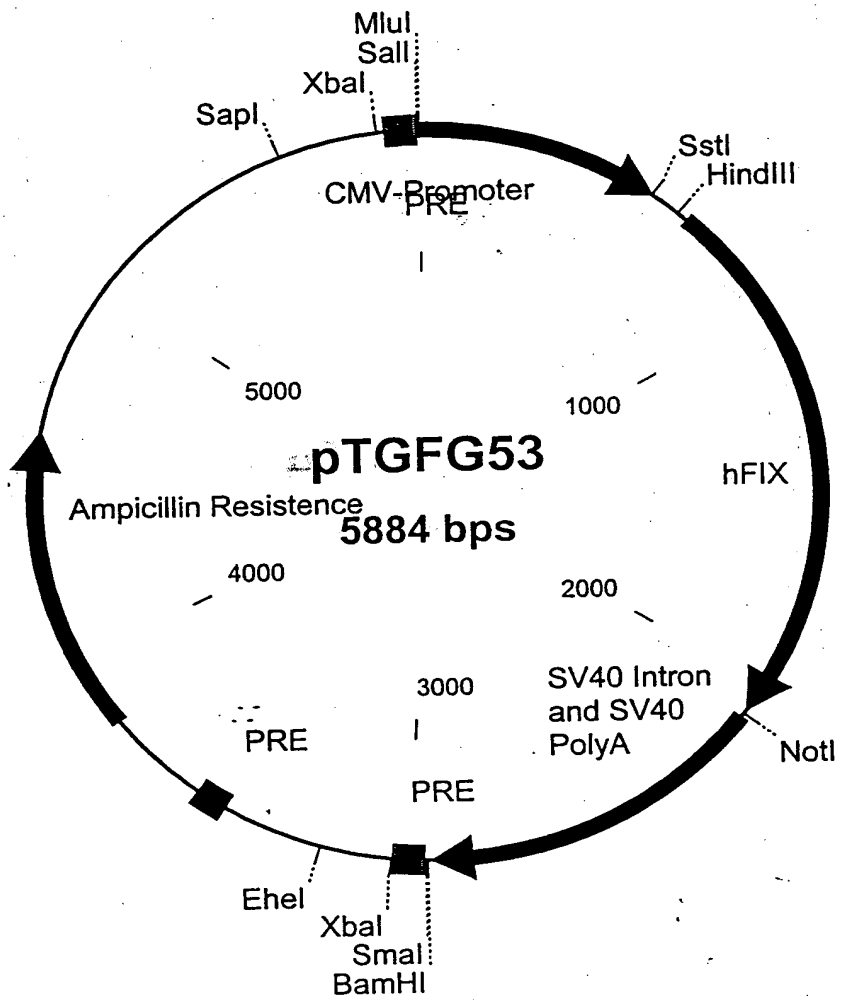

MluI
SalI
XbaI
SapI
SstI
HindIII
CMV-Promoter
PRE
1000
hFIX
2000
SV40 Intron
and SV40
PolyA
NotI
3000
PRE
PRE
EheI
XbaI
SmaI
BamHI
4000
Ampicillin Resistence
5000
pTGFG53
5884 bps

Figure 37

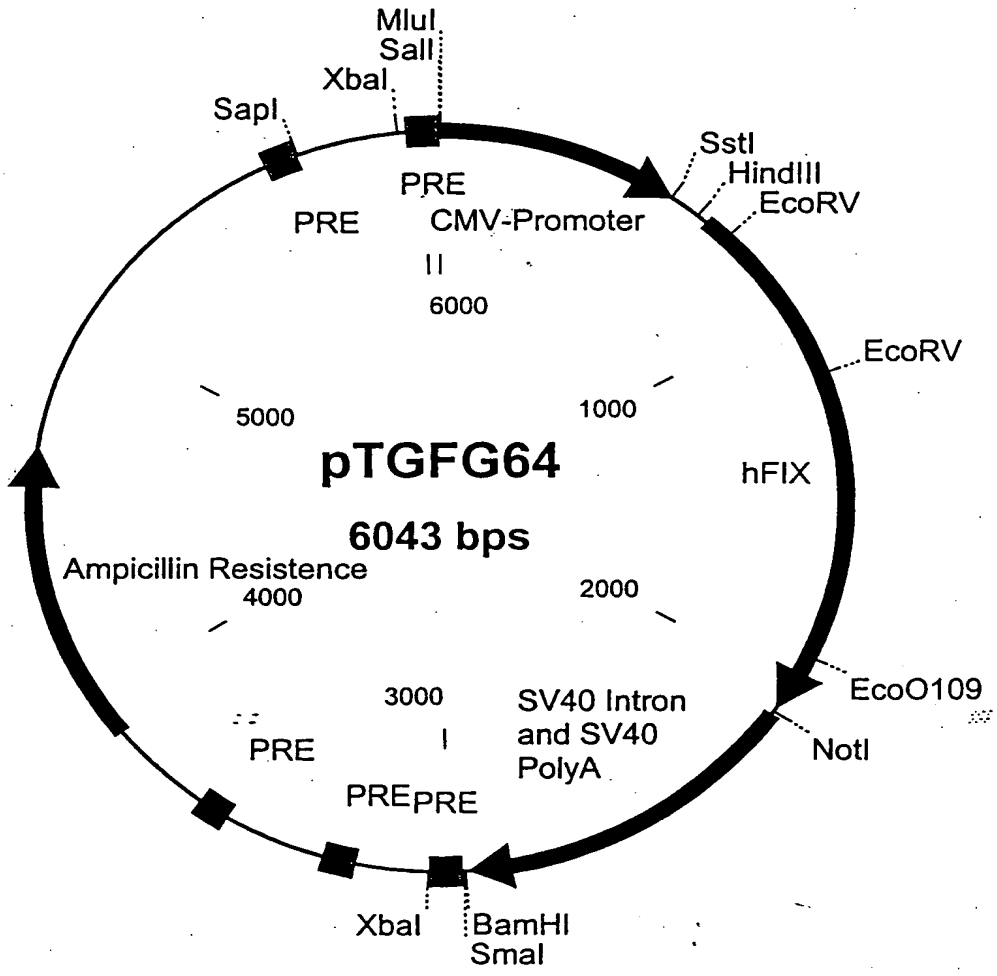

MluI
SalI
XbaI
SapI
SstI
HindIII
EcoRV
PRE
PRE
CMV-Promoter
6000
EcoRV
1000
5000
pTGFG64
hFIX
6043 bps
Ampicillin Resistence
4000
2000
EcoO109
3000
SV40 Intron
and SV40
PolyA
NotI
PRE
PREPRE
XbaI
BamHI
SmaI

Figure 38

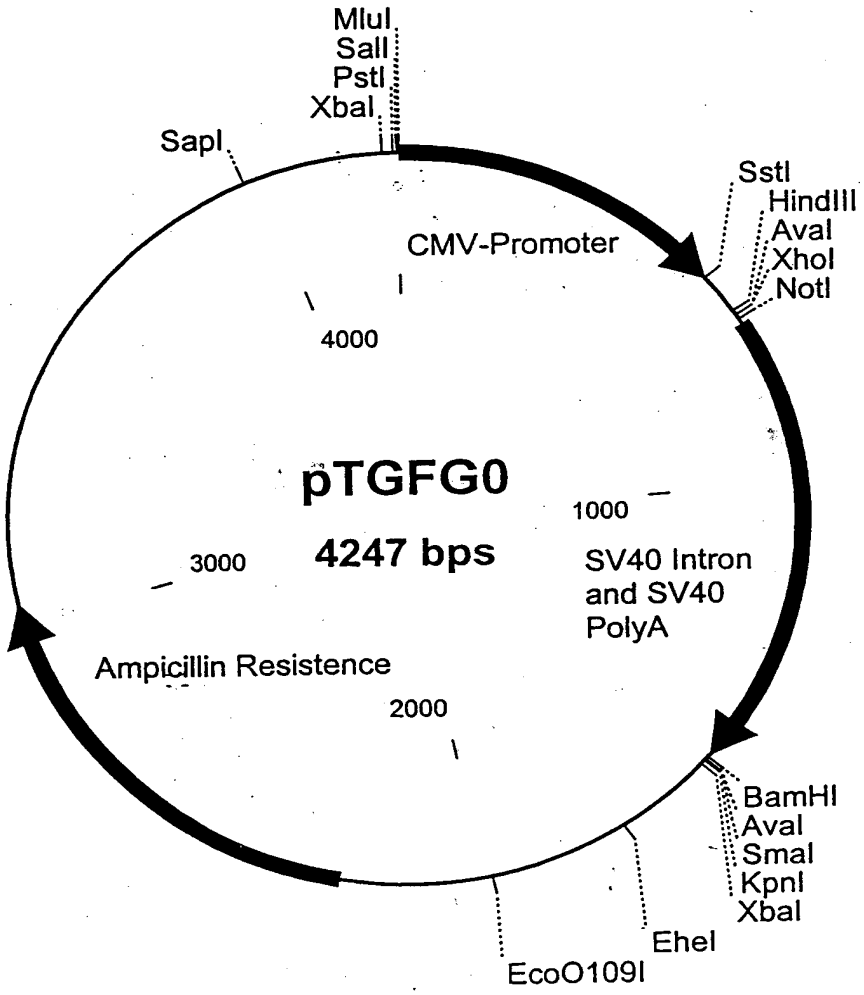

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
AvaI
XhoI
NotI
CMV-Promoter
4000
pTGFG0
4247 bps
1000
3000
SV40 Intron
and SV40
PolyA
Ampicillin Resistence
2000
BamHI
AvaI
SmaI
KpnI
XbaI
EheI
EcoO109I

Figure 39

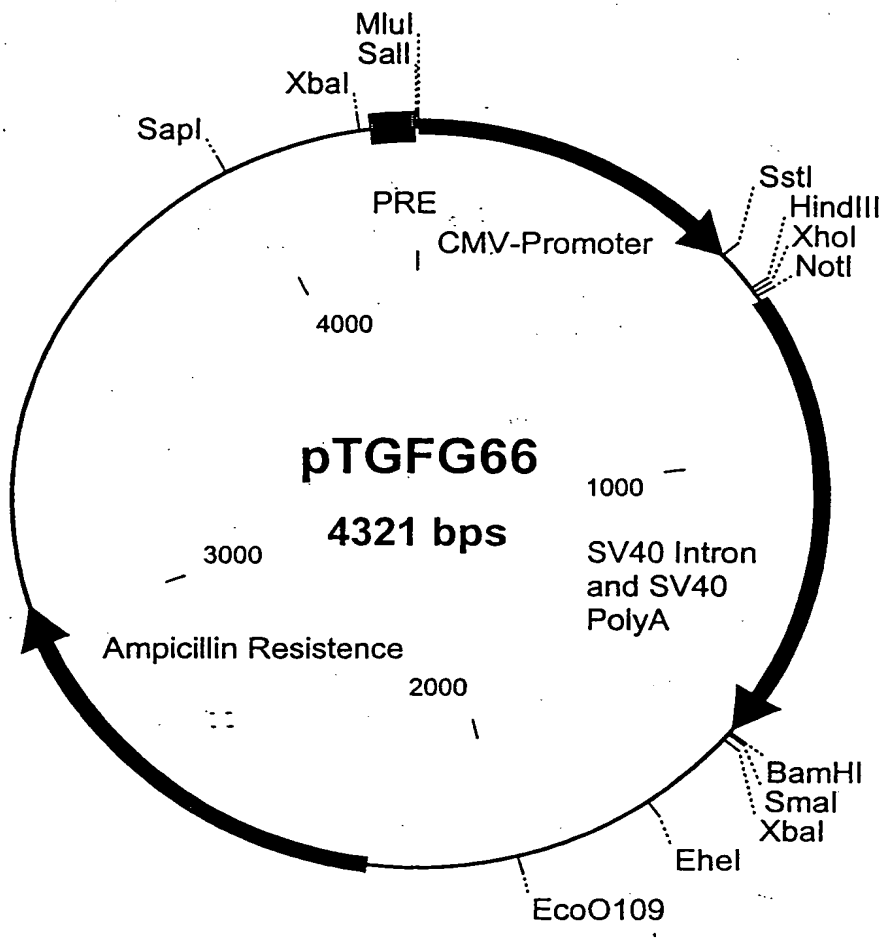

MluI
SalI
XbaI
SapI
PRE
CMV-Promoter
SstI
HindIII
XhoI
NotI
4000
pTGFG66
4321 bps
1000
3000
SV40 Intron
and SV40
PolyA
Ampicillin Resistence
2000
BamHI
SmaI
XbaI
EheI
EcoO109

Figure 40

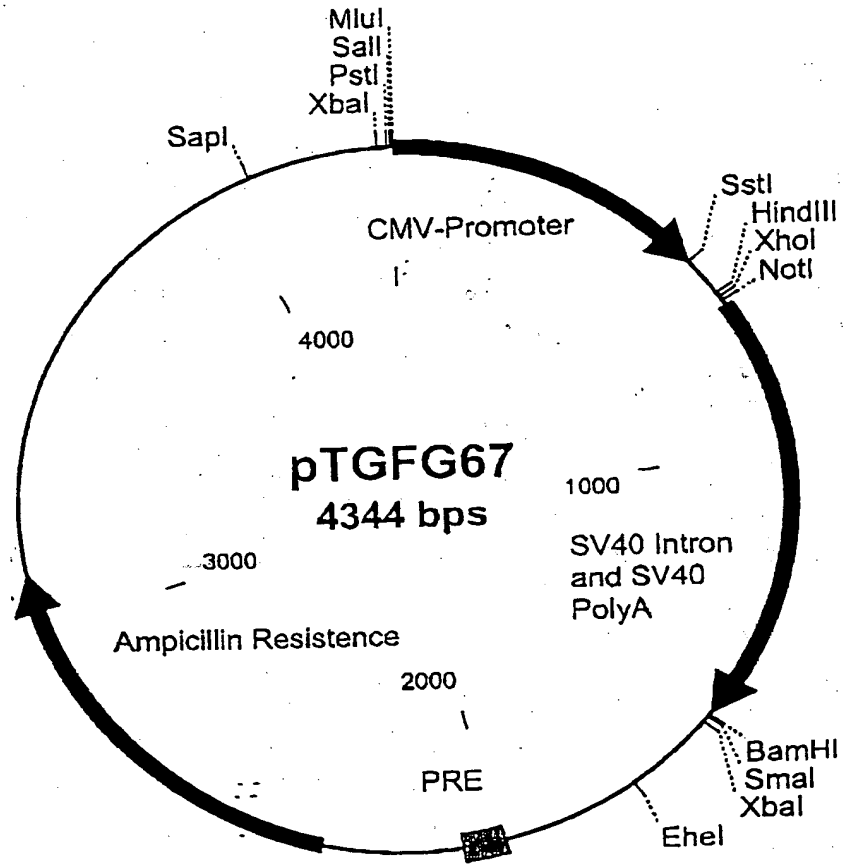

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
XhoI
NotI
CMV-Promoter
4000
pTGFG67
4344 bps
1000
SV40 Intron
and SV40
PolyA
3000
Ampicillin Resistence
2000
PRE
BamHI
SmaI
XbaI
EheI

Figure 41

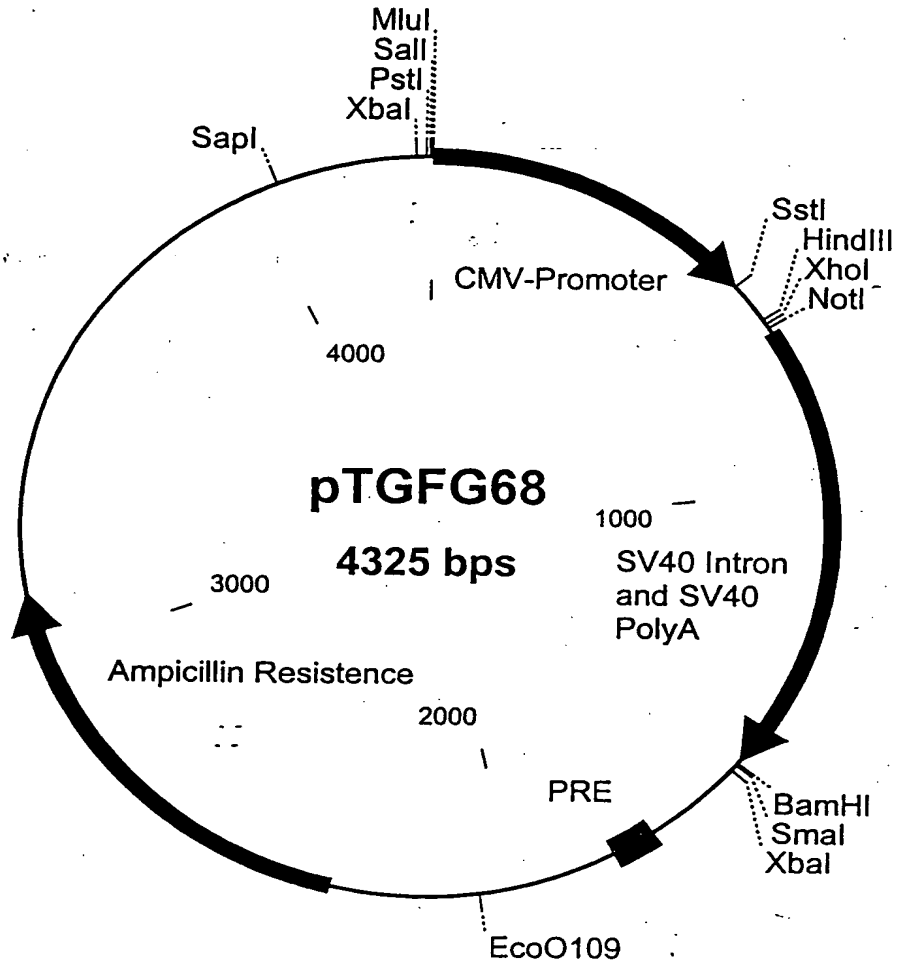

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
XhoI
NotI
CMV-Promoter
4000
pTGFG68
4325 bps
1000
SV40 Intron
and SV40
PolyA
3000
Ampicillin Resistence
2000
PRE
BamHI
SmaI
XbaI
EcoO109

Figure 42

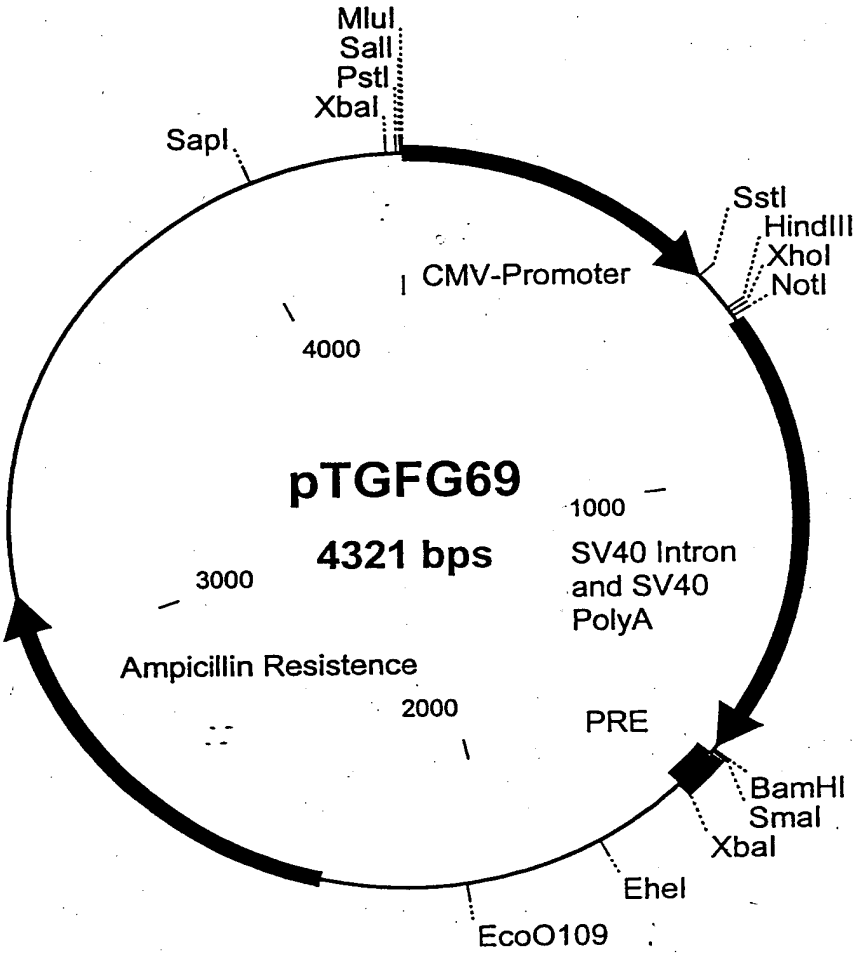

MluI
SalI
PstI
XbaI
SapI
SstI
HindIII
XhoI
NotI
CMV-Promoter
4000
pTGFG69
4321 bps
1000
SV40 Intron
and SV40
PolyA
3000
Ampicillin Resistence
2000
PRE
BamHI
SmaI
XbaI
EheI
EcoO109

Figure 43

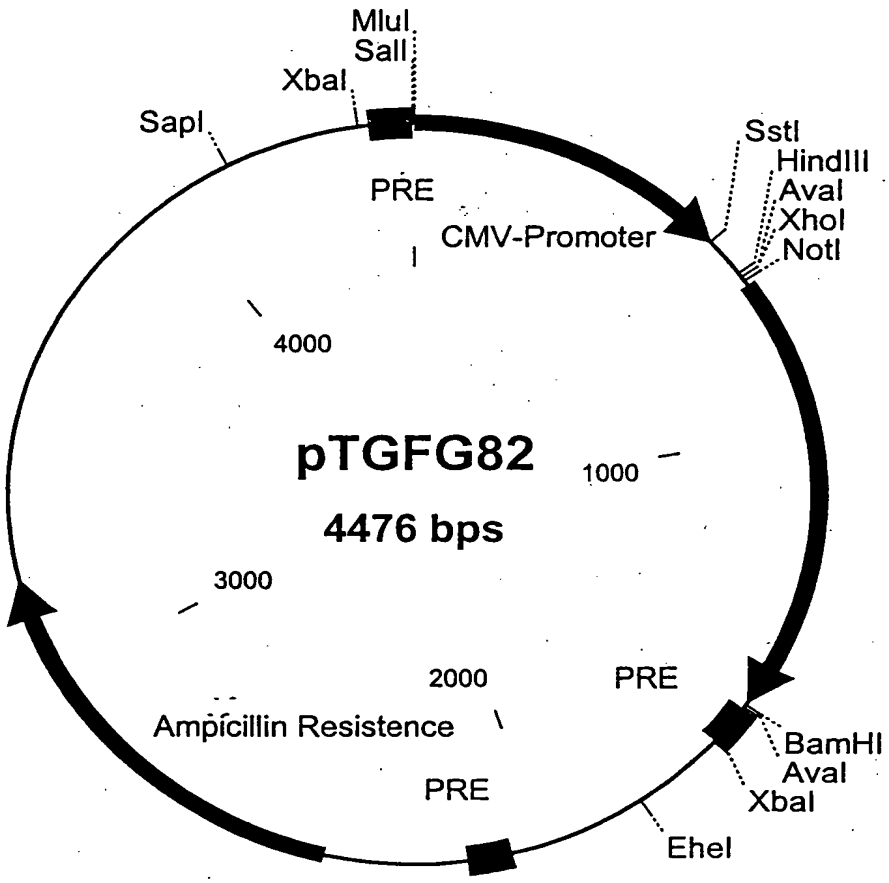
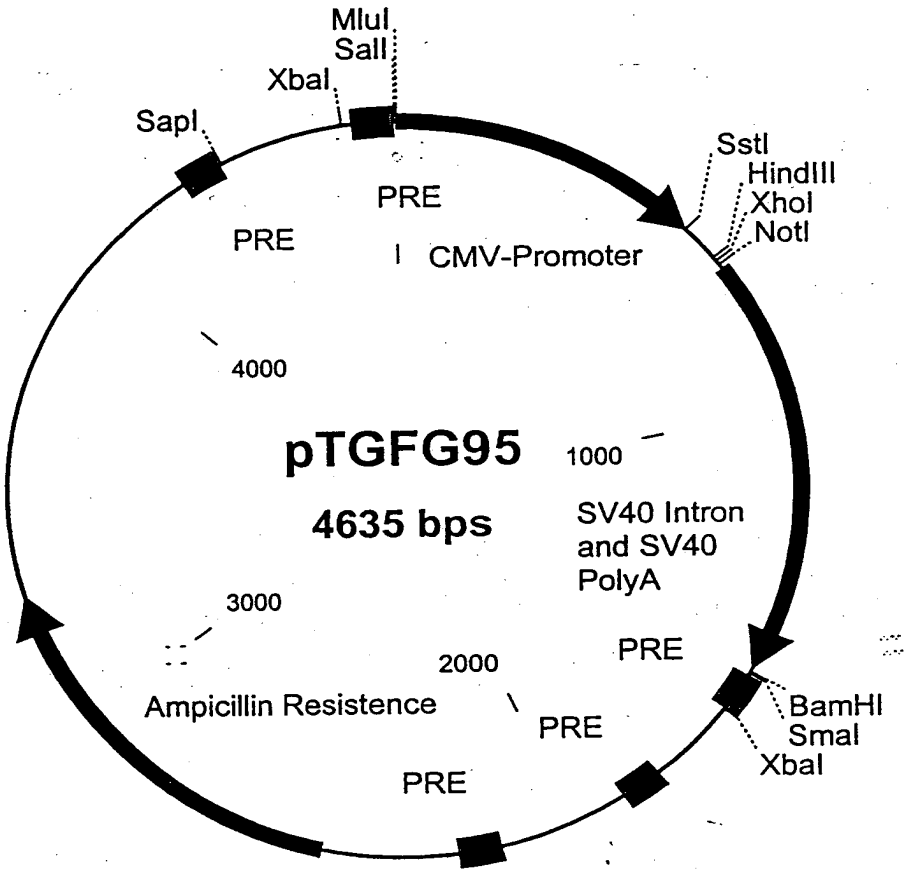
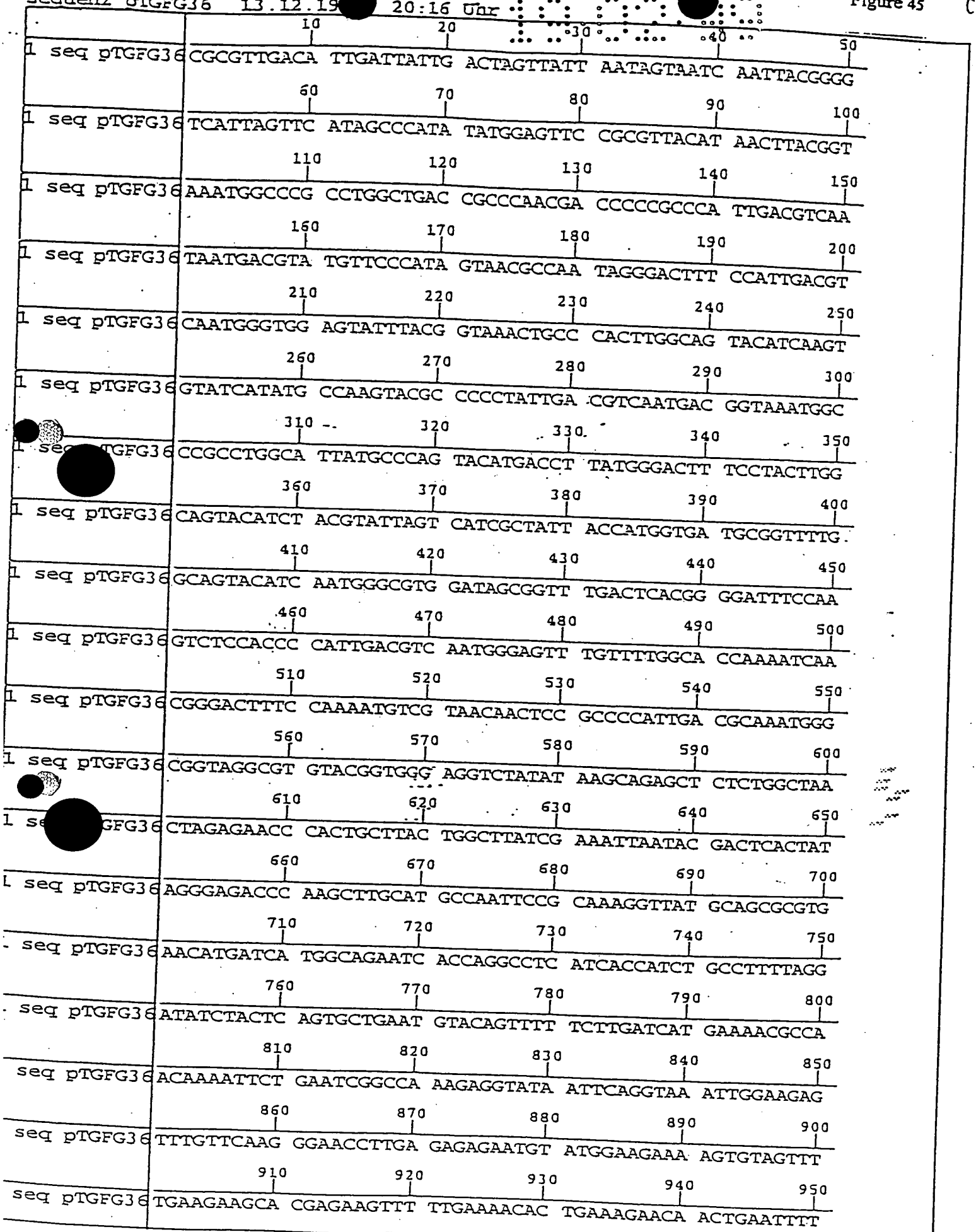

MluI
SalI
XbaI
SapI
SstI
HindIII
AvaI
XhoI
NotI
PRE
CMV-Promoter
4000
pTGFG82
4476 bps
1000
3000
2000
PRE
Ampicillin Resistence
BamHI
AvaI
XbaI
PRE
EheI

Figure 44


MluI
SalI
XbaI
SapI
SstI
HindIII
XhoI
NotI
PRE
PRE
CMV-Promoter
4000
pTGFG95
4635 bps
1000
SV40 Intron
and SV40
PolyA
3000
2000
PRE
Ampicillin Resistence
PRE
PRE
BamHI
SmaI
XbaI

Sequenz pTGFG36 13.12.19[redacted] 20:16 Uhr

Figure 45



```
                    960        970        980        990       1000
1 seq pTGFG36 GGAAGCAGTA TGTTGATGGA GATCAGTGTG AGTCCAATCC ATGTTTAAAT

                   1010       1020       1030       1040       1050
1 seq pTGFG36 GGCGGCAGTT GCAAGGATGA CATTAATTCC TATGAATGTT GGTGTCCCTT

                   1060       1070       1080       1090       1100
1 seq pTGFG36 TGGATTTGAA GGAAAGAACT GTGAATTAGA TGTAACATGT AACATTAAGA

                   1110       1120       1130       1140       1150
1 seq pTGFG36 ATGGCAGATG CGAGCAGTTT TGTAAAAATA GTGCTGATAA CAAGGTGGTT

                   1160       1170       1180       1190       1200
1 seq pTGFG36 TGCTCCTGTA CTGAGGGATA TCGACTTGCA GAAAACCAGA AGTCCTGTGA

                   1210       1220       1230       1240       1250
1 seq pTGFG36 ACCAGCAGTG CCATTTCCAT GTGGAAGAGT TTCTGTTTCA CAAACTTCTA

                   1260       1270       1280       1290       1300
1 seq pTGFG36 AGCTCACCCG TGCTGAGACT GTTTTTCCTG ATGTGGACTA TGTAAATTCT

                   1310       1320       1330       1340       1350
1 seq pTGFG36 ACTGAAGCTG AAACCATTTT GGATAACATC ACTCAAAGCA CCCAATCATT

                   1360       1370       1380       1390       1400
1 seq pTGFG36 TAATGACTTC ACTCGGGTTG TTGGTGGAGA AGATGCCAAA CCAGGTCAAT

                   1410       1420       1430       1440       1450
1 seq pTGFG36 TCCCTTGGCA GGTTGTTTTG AATGGTAAAG TTGATGCATT CTGTGGAGGC

                   1460       1470       1480       1490       1500
1 seq pTGFG36 TCTATCGTTA ATGAAAAATG GATTGTAACT GCTGCCCACT GTGTTGAAAC

                   1510       1520       1530       1540       1550
1 seq pTGFG36 TGGTGTTAAA ATTACAGTTG TCGCAGGTGA ACATAATATT GAGGAGACAG

                   1560       1570       1580       1590       1600
1 seq pTGFG36 AACATACAGA GCAAAAGCGA AATGTGATTC GAATTATTCC TCACCACAAC

                   1610       1620       1630       1640       1650
1 seq pTGFG36 TACAATGCAG CTATTAATAA GTACAACCAT GACATTGCCC TTCTGGAACT

                   1660       1670       1680       1690       1700
1 seq pTGFG36 GGACGAACCC TTAGTGCTAA ACAGCTACGT TACACCTATT TGCATTGCTG

                   1710       1720       1730       1740       1750
1 seq pTGFG36 ACAAGGAATA CACGAACATC TTCCTCAAAT TTGGATCTGG CTATGTAAGT

                   1760       1770       1780       1790       1800
1 seq pTGFG36 GGCTGGGGAA GAGTCTTCCA CAAAGGGAGA TCAGCTTTAG TTCTTCAGTA

                   1810       1820       1830       1840       1850
1 seq pTGFG36 CCTTAGAGTT CCACTTGTTG ACCGAGCCAC ATGTCTTCGA TCTACAAAGT

                   1860       1870       1880       1890       1900
1 seq pTGFG36 TCACCATCTA TAACAACATG TTCTGTGCTG GCTTCCATGA AGGAGGTAGA
```

Sequenz pTGFG36 13.12.1998 20:16 Uhr

```
                1910        1920        1930        1940        1950
L seq pTGFG36 GATTCATGTC AAGGAGATAG TGGGGGACCC CATGTTACTG AAGTGGAAGG
                1960        1970        1980        1990        2000
L seq pTGFG36 GACCAGTTTC TTAACTGGAA TTATTAGCTG GGGTGAAGAG TGTGCAATGA
                2010        2020        2030        2040        2050
L seq pTGFG36 AAGGCAAATA TGGAATATAT ACCAAGGTAT CCCGGTATGT CAACTGGATT
                2060        2070        2080        2090        2100
L seq pTGFG36 AAGGAAAAAA CAAAGCTCAC TTAATGGGAT CGGTCGAGCG GCCGCGACTC
                2110        2120        2130        2140        2150
L seq pTGFG36 TACTAGAGGA TCTTTGTGAA GGAACCTTAC TTCTGTGGTG TGACATAATT
                2160        2170        2180        2190        2200
L seq pTGFG36 GGACAAACTA CCTACAGAGA TTTAAAGCTC TAAGGTAAAT ATAAAATTTT
                2210        2220        2230        2240        2250
L seq pTGFG36 TAAGTGTATA ATGTGTTAAA CTACTGATTC TAATTGTTTG TGTATTTTAG
                2260        2270        2280        2290        2300
L seq pTGFG36 ATTCCAACCT ATGGAACTGA TGAATGGGAG CAGTGGTGGA ATGCCTTTAA
                2310        2320        2330        2340        2350
L seq pTGFG36 TGAGGAAAAC CTGTTTTGCT CAGAAGAAAT GCCATCTAGT GATGATGAGG
                2360        2370        2380        2390        2400
L seq pTGFG36 CTACTGCTGA CTCTCAACAT TCTACTCCTC CAAAAAAGAA GAGAAAGGTA
                2410        2420        2430        2440        2450
- seq pTGFG36 GAAGACCCCA AGGACTTTCC TTCAGAATTG CTAAGTTTTT TGAGTCATGC
                2460        2470        2480        2490        2500
- seq pTGFG36 TGTGTTTAGT AATAGAACTC TTGCTTGCTT TGCTATTTAC ACCACAAAGG
                2510        2520        2530        2540        2550
- seq pTGFG36 AAAAAGCTGC ACTGCTATAC AAGAAAATTA TGGAAAAATA TTCTGTAACC
                2560        2570        2580        2590        2600
- seq pTGFG36 TTTATAAGTA GGCATAACAG TTATAATCAT AACATACTGT TTTTTCTTAC
                2610        2620        2630        2640        2650
- seq pTGFG36 TCCACACAGG CATAGAGTGT CTGCTATTAA TAACTATGCT CAAAAATTGT
                2660        2670        2680        2690        2700
- seq pTGFG36 GTACCTTTAG CTTTTTAATT TGTAAAGGGG TTAATAAGGA ATATTTGATG
                2710        2720        2730        2740        2750
- seq pTGFG36 TATAGTGCCT TGACTAGAGA TCATAATCAG CCATACCACA TTTGTAGAGG
                2760        2770        2780        2790        2800
- seq pTGFG36 TTTTACTTGC TTTAAAAAAC CTCCCACACC TCCCCCTGAA CCTGAAACAT
                2810        2820        2830        2840        2850
- seq pTGFG36 AAAATGAATG CAATTGTTGT TGTTAACTTG TTTATTGCAG CTTATAATGG
```

Sequenz pTGFG36 13.12.1998 20:16 Uhr

```
                2860       2870       2880       2890       2900
1 seq pTGFG36 TTACAAATAA AGCAATAGCA TCACAAATTT CACAAATAAA GCATTTTTTT
                2910       2920       2930       2940       2950
1 seq pTGFG36 CACTGCATTC TAGTTGTGGT TTGTCCAAAC TCATCAATGT ATCTTATCAT
                2960       2970       2980       2990       3000
1 seq pTGFG36 GTCTGGATCC CCGGGTACCC TCTAGAGCGA ATTAATTCAC TGGCCGTCGT
                3010       3020       3030       3040       3050
1 seq pTGFG36 TTTACAACGT CGTGACTGGG AAAACCCTGG CGTTACCCAA CTTAATCGCC
                3060       3070       3080       3090       3100
1 seq pTGFG36 TTGCAGCACA TCCCCCTTTC GCCAGCTGGC GTAATAGCGA AGAGGCCCGC
                3110       3120       3130       3140       3150
1 seq pTGFG36 ACCGATCGCC CTTCCCAACA GTTGCGCAGC CTGAATGGCG AATGGCGCCT
                3160       3170       3180       3190       3200
1 seq pTGFG36 GATGCGGTAT TTTCTCCTTA CGCATCTGTG CGGTATTTCA CACCGCATAT
                3210       3220       3230       3240       3250
1 seq pTGFG36 GGTGCACTCT CAGTACAATC TGCTCTGATG CCGCATAGTT AAGCCAGCCC
                3260       3270       3280       3290       3300
1 seq pTGFG36 CGACACCCGC CAACACCCGC TGACGCGCCC TGACGGGCTT GTCTGCTCCC
                3310       3320       3330       3340       3350
1 seq pTGFG36 GGCATCCGCT TACAGACAAG CTGTGACCGT CTCCGGGAGC TGCATGTGTC
                3360       3370       3380       3390       3400
1 seq pTGFG36 AGAGGTTTTC ACCGTCATCA CCGAAACGCG CGAGACGAAA GGGGGGGTAC
                3410       3420       3430       3440       3450
1 seq pTGFG36 CAGCTTCGTA GCTAGAACAT CATGTTCTGG GATATCAGCT TCGTAGCTAG
                3460       3470       3480       3490       3500
1 seq pTGFG36 AACATCATGT TCTGGTACCC CCCTCGTGAT ACGCCTATTT TTATAGGTTA
                3510       3520       3530       3540       3550
1 seq pTGFG36 ATGTCATGAT AATAATGGTT TCTTAGACGT CAGGTGGCAC TTTTCGGGGA
                3560       3570       3580       3590       3600
1 seq pTGFG36 AATGTGCGCG GAACCCCTAT TTGTTTATTT TTCTAAATAC ATTCAAATAT
                3610       3620       3630       3640       3650
1 seq pTGFG36 GTATCCGCTC ATGAGACAAT AACCCTGATA AATGCTTCAA TAATATTGAA
                3660       3670       3680       3690       3700
1 seq pTGFG36 AAAGGAAGAG TATGAGTATT CAACATTTCC GTGTCGCCCT TATTCCCTTT
                3710       3720       3730       3740       3750
1 seq pTGFG36 TTTGCGGCAT TTTGCCTTCC TGTTTTTGCT CACCCAGAAA CGCTGGTGAA
                3760       3770       3780       3790       3800
1 seq pTGFG36 AGTAAAAGAT GCTGAAGATC AGTTGGGTGC ACGAGTGGGT TACATCGAAC
```

```
                3810        3820        3830        3840        3850
1 seq pTGFG36 TGGATCTCAA CAGCGGTAAG ATCCTTGAGA GTTTTCGCCC CGAAGAACGT
                3860        3870        3880        3890        3900
1 seq pTGFG36 TTTCCAATGA TGAGCACTTT TAAAGTTCTG CTATGTGGCG CGGTATTATC
                3910        3920        3930        3940        3950
1 seq pTGFG36 CCGTATTGAC GCCGGGCAAG AGCAACTCGG TCGCCGCATA CACTATTCTC
                3960        3970        3980        3990        4000
1 seq pTGFG36 AGAATGACTT GGTTGAGTAC TCACCAGTCA CAGAAAAGCA TCTTACGGAT
                4010        4020        4030        4040        4050
1 seq pTGFG36 GGCATGACAG TAAGAGAATT ATGCAGTGCT GCCATAACCA TGAGTGATAA
                4060        4070        4080        4090        4100
1 seq pTGFG36 CACTGCGGCC AACTTACTTC TGACAACGAT CGGAGGACCG AAGGAGCTAA
                4110        4120        4130        4140        4150
1 se[redacted]FG36 CCGCTTTTTT GCACAACATG GGGGATCATG TAACTCGCCT TGATCGTTGG
                4160        4170        4180        4190        4200
1 seq pTGFG36 GAACCGGAGC TGAATGAAGC CATACCAAAC GACGAGCGTG ACACCACGAT
                4210        4220        4230        4240        4250
1 seq pTGFG36 GCCTGTAGCA ATGGCAACAA CGTTGCGCAA ACTATTAACT GGCGAACTAC
                4260        4270        4280        4290        4300
1 seq pTGFG36 TTACTCTAGC TTCCCGGCAA CAATTAATAG ACTGGATGGA GGCGGATAAA
                4310        4320        4330        4340        4350
1 seq pTGFG36 GTTGCAGGAC CACTTCTGCG CTCGGCCCTT CCGGCTGGCT GGTTTATTGC
                4360        4370        4380        4390        4400
1 [redacted] pTGFG36 TGATAAATCT GGAGCCGGTG AGCGTGGGTC TCGCGGTATC ATTGCAGCAC
                4410        4420        4430        4440        4450
1 se[redacted]GFG36 TGGGGCCAGA TGGTAAGCCC TCCCGTATCG TAGTTATCTA CACGACGGGG
                4460        4470        4480        4490        4500
1 seq pTGFG36 AGTCAGGCAA CTATGGATGA ACGAAATAGA CAGATCGCTG AGATAGGTGC
                4510        4520        4530        4540        4550
1 seq pTGFG36 CTCACTGATT AAGCATTGGT AACTGTCAGA CCAAGTTTAC TCATATATAC
                4560        4570        4580        4590        4600
1 seq pTGFG36 TTTAGATTGA TTTAAAACTT CATTTTTAAT TTAAAAGGAT CTAGGTGAAG
                4610        4620        4630        4640        4650
1 seq pTGFG36 ATCCTTTTTG ATAATCTCAT GACCAAAATC CCTTAACGTG AGTTTTCGTT
                4660        4670        4680        4690        4700
1 seq pTGFG36 CCACTGAGCG TCAGACCCCG TAGAAAAGAT CAAAGGATCT TCTTGAGATC
                4710        4720        4730        4740        4750
1 seq pTGFG36 CTTTTTTTCT GCGCGTAATC TGCTGCTTGC AAACAAAAAA ACCACCGCTA
```

Sequenz pTGFG36 13.12.1998 20:16 Uhr

```
                4760        4770        4780        4790        4800
1 seq pTGFG36 CCAGCGGTGG TTTGTTTGCC GGATCAAGAG CTACCAACTC TTTTTCCGAA
                4810        4820        4830        4840        4850
1 seq pTGFG36 GGTAACTGGC TTCAGCAGAG CGCAGATACC AAATACTGTT CTTCTAGTGT
                4860        4870        4880        4890        4900
1 seq pTGFG36 AGCCGTAGTT AGGCCACCAC TTCAAGAACT CTGTAGCACC GCCTACATAC
                4910        4920        4930        4940        4950
1 seq pTGFG36 CTCGCTCTGC TAATCCTGTT ACCAGTGGCT GCTGCCAGTG GCGATAAGTC
                4960        4970        4980        4990        5000
1 seq pTGFG36 GTGTCTTACC GGGTTGGACT CAAGACGATA GTTACCGGAT AAGGCGCAGC
                5010        5020        5030        5040        5050
1 seq pTGFG36 GGTCGGGCTG AACGGGGGGT TCGTGCACAC AGCCCAGCTT GGAGCGAACG
                5060        5070        5080        5090        5100
1 seq pTGFG36 ACCTACACCG AACTGAGATA CCTACAGCGT GAGCTATGAG AAAGCGCCAC
                5110        5120        5130        5140        5150
1 seq pTGFG36 GCTTCCCGAA GGGAGAAAGG CGGACAGGTA TCCGGTAAGC GGCAGGGTCG
                5160        5170        5180        5190        5200
1 seq pTGFG36 GAACAGGAGA GCGCACGAGG GAGCTTCCAG GGGGAAACGC CTGGTATCTT
                5210        5220        5230        5240        5250
1 seq pTGFG36 TATAGTCCTG TCGGGTTTCG CCACCTCTGA CTTGAGCGTC GATTTTTGTG
                5260        5270        5280        5290        5300
1 seq pTGFG36 ATGCTCGTCA GGGGGGCGGA GCCTATGGAA AAACGCCAGC AACGCGGCCT
                5310        5320        5330        5340        5350
1 seq pTGFG36 TTTTACGGTT CCTGGCCTTT TGCTGGCCTT TTGCTCACAT GTTCTTTCCT
                5360        5370        5380        5390        5400
1 seq pTGFG36 GCGTTATCCC CTGATTCTGT GGATAACCGT ATTACCGCCT TTGAGTGAGC
                5410        5420        5430        5440        5450
1 seq pTGFG36 TGATACCGCT CGCCGCAGCC GAACGACCGA GCGCAGCGAG TCAGTGAGCG
                5460        5470        5480        5490        5500
1 seq pTGFG36 AGGAAGCGGA AGAGCGCCCA ATACGCAAAC CGCCTCTCCC CGCGCGTTGG
                5510        5520        5530        5540        5550
1 seq pTGFG36 CCGATTCATT AATGCAGCTG GCACGACAGG TTTCCCGACT GGAAAGCGGG
                5560        5570        5580        5590        5600
1 seq pTGFG36 CAGTGAGCGC AACGCAATTA ATGTGAGTTA GCTCACTCAT TAGGCACCCC
                5610        5620        5630        5640        5650
1 seq pTGFG36 AGGCTTTACA CTTTATGCTT CCGGCTCGTA TGTTGTGTGG AATTGTGAGC
                5660        5670        5680        5690        5700
1 seq pTGFG36 GGATAACAAT TTCACACAGG AAACAGCTAT GACCATGATT ACGCCAAGCT
```

Sequenz pTGFG36 13.12.1998 20:16 Uhr

	5710	5720	5730	5740	5750
1 seq pTGFG36	CTCTAGAGCT	CTAGAGCTCT	AGAGCTCTAG	AGAGCTTGCA	TGCCTGCAGG

	5760	5770	5780	5790	5800
1 seq pTGFG36	TCG				

Sequenz pTGFG67 13.[illegible].1998 21:02 Uhr

```
                    10        20        30        40        50
1 seq pTGFG67 CGCGTTGACA TTGATTATTG ACTAGTTATT AATAGTAATC AATTACGGGG
                    60        70        80        90       100
1 seq pTGFG67 TCATTAGTTC ATAGCCCATA TATGGAGTTC CGCGTTACAT AACTTACGGT
                   110       120       130       140       150
1 seq pTGFG67 AAATGGCCCG CCTGGCTGAC CGCCCAACGA CCCCCGCCCA TTGACGTCAA
                   160       170       180       190       200
1 seq pTGFG67 TAATGACGTA TGTTCCCATA GTAACGCCAA TAGGGACTTT CCATTGACGT
                   210       220       230       240       250
1 seq pTGFG67 CAATGGGTGG AGTATTTACG GTAAACTGCC CACTTGGCAG TACATCAAGT
                   260       270       280       290       300
1 seq pTGFG67 GTATCATATG CCAAGTACGC CCCCTATTGA CGTCAATGAC GGTAAATGGC
                   310       320       330       340       350
1 seq pTGFG67 CCGCCTGGCA TTATGCCCAG TACATGACCT TATGGGACTT TCCTACTTGG
                   360       370       380       390       400
1 seq pTGFG67 CAGTACATCT ACGTATTAGT CATCGCTATT ACCATGGTGA TGCGGTTTTG
                   410       420       430       440       450
1 seq pTGFG67 GCAGTACATC AATGGGCGTG GATAGCGGTT TGACTCACGG GGATTTCCAA
                   460       470       480       490       500
1 seq pTGFG67 GTCTCCACCC CATTGACGTC AATGGGAGTT TGTTTTGGCA CCAAAATCAA
                   510       520       530       540       550
1 seq pTGFG67 CGGGACTTTC CAAAATGTCG TAACAACTCC GCCCCATTGA CGCAAATGGG
                   560       570       580       590       600
1 seq pTGFG67 CGGTAGGCGT GTACGGTGGG AGGTCTATAT AAGCAGAGCT CTCTGGCTAA
                   610       620       630       640       650
1 seq pTGFG67 CTAGAGAACC CACTGCTTAC TGGCTTATCG AAATTAATAC GACTCACTAT
                   660       670       680       690       700
1 seq pTGFG67 AGGGAGACCC AAGCTTGACC TCGAGCAAGC GGCCGCGACT CTACTAGAGG
                   710       720       730       740       750
1 seq pTGFG67 ATCTTTGTGA AGGAACCTTA CTTCTGTGGT GTGACATAAT TGGACAAACT
                   760       770       780       790       800
1 seq pTGFG67 ACCTACAGAG ATTTAAAGCT CTAAGGTAAA TATAAAATTT TTAAGTGTAT
                   810       820       830       840       850
1 seq pTGFG67 AATGTGTTAA ACTACTGATT CTAATTGTTT GTGTATTTTA GATTCCAACC
                   860       870       880       890       900
1 seq pTGFG67 TATGGAACTG ATGAATGGGA GCAGTGGTGG AATGCCTTTA ATGAGGAAAA
                   910       920       930       940       950
1 seq pTGFG67 CCTGTTTTGC TCAGAAGAAA TGCCATCTAG TGATGATGAG GCTACTGCTG
```

Sequenz pTGFG67 13.12.1998 21:02 Uhr

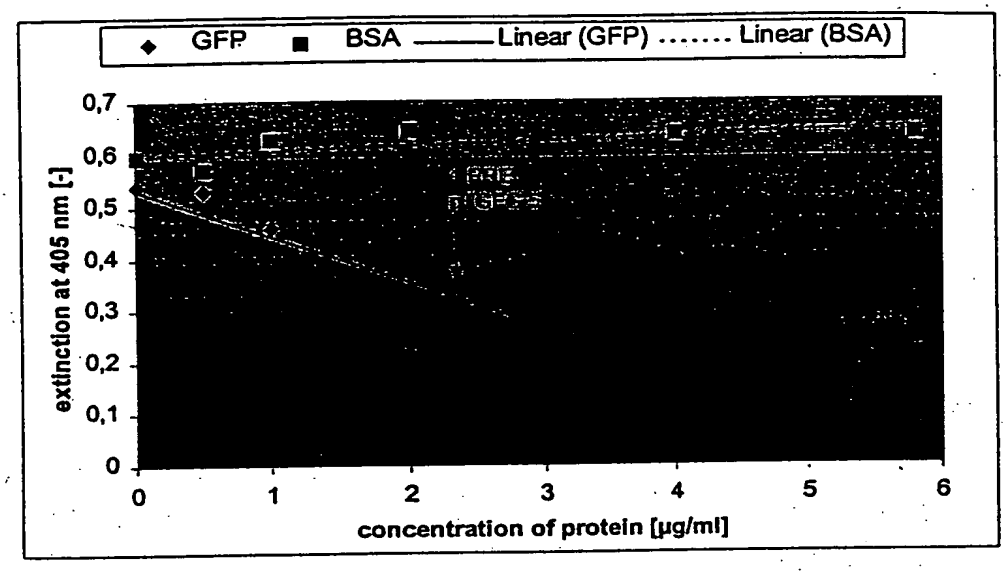
	960	970	980	990	1000
1 seq pTGFG67	ACTCTCAACA	TTCTACTCCT	CCAAAAAAGA	AGAGAAAGGT	AGAAGACCCC
	1010	1020	1030	1040	1050
1 seq pTGFG67	AAGGACTTTC	CTTCAGAATT	GCTAAGTTTT	TTGAGTCATG	CTGTGTTTAG
	1060	1070	1080	1090	1100
1 seq pTGFG67	TAATAGAACT	CTTGCTTGCT	TTGCTATTTA	CACCACAAAG	GAAAAAGCTG
	1110	1120	1130	1140	1150
1 seq pTGFG67	CACTGCTATA	CAAGAAAATT	ATGGAAAAAT	ATTCTGTAAC	CTTTATAAGT
	1160	1170	1180	1190	1200
1 seq pTGFG67	AGGCATAACA	GTTATAATCA	TAACATACTG	TTTTTTCTTA	CTCCACACAG
	1210	1220	1230	1240	1250
1 seq pTGFG67	GCATAGAGTG	TCTGCTATTA	ATAACTATGC	TCAAAAATTG	TGTACCTTTA
	1260	1270	1280	1290	1300
1 s[redacted]GFG67	GCTTTTTAAT	TTGTAAAGGG	GTTAATAAGG	AATATTTGAT	GTATAGTGCC
	1310	1320	1330	1340	1350
1 seq pTGFG67	TTGACTAGAG	ATCATAATCA	GCCATACCAC	ATTTGTAGAG	GTTTTACTTG
	1360	1370	1380	1390	1400
1 seq pTGFG67	CTTTAAAAAA	CCTCCCACAC	CTCCCCCTGA	ACCTGAAACA	TAAAATGAAT
	1410	1420	1430	1440	1450
1 seq pTGFG67	GCAATTGTTG	TTGTTAACTT	GTTTATTGCA	GCTTATAATG	GTTACAAATA
	1460	1470	1480	1490	1500
1 seq pTGFG67	AAGCAATAGC	ATCACAAATT	TCACAAATAA	AGCATTTTTT	TCACTGCATT
	1510	1520	1530	1540	1550
1 [redacted]eq pTGFG67	CTAGTTGTGG	TTTGTCCAAA	CTCATCAATG	TATCTTATCA	TGTCTGGATC
	1560	1570	1580	1590	1600
1 s[redacted] pTGFG67	CCCGGGTACC	CTCTAGAGCG	AATTAATTCA	CTGGCCGTCG	TTTTACAACG
	1610	1620	1630	1640	1650
1 seq pTGFG67	TCGTGACTGG	GAAAACCCTG	GCGTTACCCA	ACTTAATCGC	CTTGCAGCAC
	1660	1670	1680	1690	1700
1 seq pTGFG67	ATCCCCCTTT	CGCCAGCTGG	CGTAATAGCG	AAGAGGCCCG	CACCGATCGC
	1710	1720	1730	1740	1750
1 seq pTGFG67	CCTTCCCAAC	AGTTGCGCAG	CCTGAATGGC	GAATGGCGCC	TGATGCGGTA
	1760	1770	1780	1790	1800
1 seq pTGFG67	TTTTCTCCTT	ACGCATCTGT	GCGGTATTTC	ACACCGCATA	TGGTGCACTC
	1810	1820	1830	1840	1850
1 seq pTGFG67	TCAGTACAAT	CTGCTCTGAT	GCCGCATAGT	TAAGCCAGCC	CCGACACCCG
	1860	1870	1880	1890	1900
1 seq pTGFG67	CCAACACCCG	CTGACGCGCC	CTGACGGGCT	TGTCTGCTCC	CGGCATCCGC

```
                    1910       1920       1930       1940       1950
1 seq pTGFG67 TTACAGACAA GCTGTGACCG TCTCCGGGAG CTGCATGTGT CAGAGGTTTT
                    1960       1970       1980       1990       2000
1 seq pTGFG67 CACCGTCATC ACCGAAACGC GCGAGACGAA AGGGGGGGTA CCAGCTTCGT
                    2010       2020       2030       2040       2050
1 seq pTGFG67 AGCTAGAACA TCATGTTCTG GGATATCAGC TTCGTAGCTA GAACATCATG
                    2060       2070       2080       2090       2100
1 seq pTGFG67 TTCTGGTACC CCCCTCGTGA TACGCCTATT TTTATAGGTT AATGTCATGA
                    2110       2120       2130       2140       2150
1 seq pTGFG67 TAATAATGGT TTCTTAGACG TCAGGTGGCA CTTTTCGGGG AAATGTGCGC
                    2160       2170       2180       2190       2200
1 seq pTGFG67 GGAACCCCTA TTTGTTTATT TTTCTAAATA CATTCAAATA TGTATCCGCT
                    2210       2220       2230       2240       2250
1 se[redacted]GFG67 CATGAGACAA TAACCCTGAT AAATGCTTCA ATAATATTGA AAAAGGAAGA
                    2260       2270       2280       2290       2300
1 seq pTGFG67 GTATGAGTAT TCAACATTTC CGTGTCGCCC TTATTCCCTT TTTTGCGGCA
                    2310       2320       2330       2340       2350
1 seq pTGFG67 TTTTGCCTTC CTGTTTTTGC TCACCCAGAA ACGCTGGTGA AAGTAAAAGA
                    2360       2370       2380       2390       2400
1 seq pTGFG67 TGCTGAAGAT CAGTTGGGTG CACGAGTGGG TTACATCGAA CTGGATCTCA
                    2410       2420       2430       2440       2450
1 seq pTGFG67 ACAGCGGTAA GATCCTTGAG AGTTTTCGCC CCGAAGAACG TTTTCCAATG
                    2460       2470       2480       2490       2500
1 seq pTGFG67 ATGAGCACTT TTAAAGTTCT GCTATGTGGC GCGGTATTAT CCCGTATTGA
                    2510       2520       2530       2540       2550
1 s[redacted]GFG67 CGCCGGGCAA GAGCAACTCG GTCGCCGCAT ACACTATTCT CAGAATGACT
                    2560       2570       2580       2590       2600
1 seq pTGFG67 TGGTTGAGTA CTCACCAGTC ACAGAAAAGC ATCTTACGGA TGGCATGACA
                    2610       2620       2630       2640       2650
1 seq pTGFG67 GTAAGAGAAT TATGCAGTGC TGCCATAACC ATGAGTGATA ACACTGCGGC
                    2660       2670       2680       2690       2700
1 seq pTGFG67 CAACTTACTT CTGACAACGA TCGGAGGACC GAAGGAGCTA ACCGCTTTTT
                    2710       2720       2730       2740       2750
1 seq pTGFG67 TGCACAACAT GGGGGATCAT GTAACTCGCC TTGATCGTTG GGAACCGGAG
                    2760       2770       2780       2790       2800
1 seq pTGFG67 CTGAATGAAG CCATACCAAA CGACGAGCGT GACACCACGA TGCCTGTAGC
                    2810       2820       2830       2840       2850
1 seq pTGFG67 AATGGCAACA ACGTTGCGCA AACTATTAAC TGGCGAACTA CTTACTCTAG
```

	2860	2870	2880	2890	2900
1 seq pTGFG67	CTTCCCGGCA	ACAATTAATA	GACTGGATGG	AGGCGGATAA	AGTTGCAGGA
	2910	2920	2930	2940	2950
1 seq pTGFG67	CCACTTCTGC	GCTCGGCCCT	TCCGGCTGGC	TGGTTTATTG	CTGATAAATC
	2960	2970	2980	2990	3000
1 seq pTGFG67	TGGAGCCGGT	GAGCGTGGGT	CTCGCGGTAT	CATTGCAGCA	CTGGGGCCAG
	3010	3020	3030	3040	3050
1 seq pTGFG67	ATGGTAAGCC	CTCCCGTATC	GTAGTTATCT	ACACGACGGG	GAGTCAGGCA
	3060	3070	3080	3090	3100
1 seq pTGFG67	ACTATGGATG	AACGAAATAG	ACAGATCGCT	GAGATAGGTG	CCTCACTGAT
	3110	3120	3130	3140	3150
1 seq pTGFG67	TAAGCATTGG	TAACTGTCAG	ACCAAGTTTA	CTCATATATA	CTTTAGATTG
	3160	3170	3180	3190	3200
1 seq pTGFG67	ATTTAAAACT	TCATTTTTAA	TTTAAAAGGA	TCTAGGTGAA	GATCCTTTTT
	3210	3220	3230	3240	3250
1 seq pTGFG67	GATAATCTCA	TGACCAAAAT	CCCTTAACGT	GAGTTTTCGT	TCCACTGAGC
	3260	3270	3280	3290	3300
1 seq pTGFG67	GTCAGACCCC	GTAGAAAAGA	TCAAAGGATC	TTCTTGAGAT	CCTTTTTTTC
	3310	3320	3330	3340	3350
1 seq pTGFG67	TGCGCGTAAT	CTGCTGCTTG	CAAACAAAAA	AACCACCGCT	ACCAGCGGTG
	3360	3370	3380	3390	3400
1 seq pTGFG67	GTTTGTTTGC	CGGATCAAGA	GCTACCAACT	CTTTTTCCGA	AGGTAACTGG
	3410	3420	3430	3440	3450
1 seq pTGFG67	CTTCAGCAGA	GCGCAGATAC	CAAATACTGT	TCTTCTAGTG	TAGCCGTAGT
	3460	3470	3480	3490	3500
1 seq pTGFG67	TAGGCCACCA	CTTCAAGAAC	TCTGTAGCAC	CGCCTACATA	CCTCGCTCTG
	3510	3520	3530	3540	3550
1 seq pTGFG67	CTAATCCTGT	TACCAGTGGC	TGCTGCCAGT	GGCGATAAGT	CGTGTCTTAC
	3560	3570	3580	3590	3600
1 seq pTGFG67	CGGGTTGGAC	TCAAGACGAT	AGTTACCGGA	TAAGGCGCAG	CGGTCGGGCT
	3610	3620	3630	3640	3650
1 seq pTGFG67	GAACGGGGGG	TTCGTGCACA	CAGCCCAGCT	TGGAGCGAAC	GACCTACACC
	3660	3670	3680	3690	3700
1 seq pTGFG67	GAACTGAGAT	ACCTACAGCG	TGAGCTATGA	GAAAGCGCCA	CGCTTCCCGA
	3710	3720	3730	3740	3750
1 seq pTGFG67	AGGGAGAAAG	GCGGACAGGT	ATCCGGTAAG	CGGCAGGGTC	GGAACAGGAG
	3760	3770	3780	3790	3800
1 seq pTGFG67	AGCGCACGAG	GGAGCTTCCA	GGGGGAAACG	CCTGGTATCT	TTATAGTCCT

```
                    3810       3820       3830       3840       3850
1 seq pTGFG67 GTCGGGTTTC GCCACCTCTG ACTTGAGCGT CGATTTTTGT GATGCTCGTC

                    3860       3870       3880       3890       3900
1 seq pTGFG67 AGGGGGGCGG AGCCTATGGA AAAACGCCAG CAACGCGGCC TTTTTACGGT

                    3910       3920       3930       3940       3950
1 seq pTGFG67 TCCTGGCCTT TTGCTGGCCT TTTGCTCACA TGTTCTTTCC TGCGTTATCC

                    3960       3970       3980       3990       4000
1 seq pTGFG67 CCTGATTCTG TGGATAACCG TATTACCGCC TTTGAGTGAG CTGATACCGC

                    4010       4020       4030       4040       4050
1 seq pTGFG67 TCGCCGCAGC CGAACGACCG AGCGCAGCGA GTCAGTGAGC GAGGAAGCGG

                    4060       4070       4080       4090       4100
1 seq pTGFG67 AAGAGCGCCC AATACGCAAA CCGCCTCTCC CCGCGCGTTG GCCGATTCAT

                    4110       4120       4130       4140       4150
1 se[redacted]GFG67 TAATGCAGCT GGCACGACAG GTTTCCCGAC TGGAAAGCGG GCAGTGAGCG

                    4160       4170       4180       4190       4200
1 seq pTGFG67 CAACGCAATT AATGTGAGTT AGCTCACTCA TTAGGCACCC CAGGCTTTAC

                    4210       4220       4230       4240       4250
1 seq pTGFG67 ACTTTATGCT TCCGGCTCGT ATGTTGTGTG GAATTGTGAG CGGATAACAA

                    4260       4270       4280       4290       4300
1 seq pTGFG67 TTTCACACAG GAAACAGCTA TGACCATGAT TACGCCAAGC TCTCTAGAGC

                    4310       4320       4330       4340       4350
1 seq pTGFG67 TCTAGAGCTC TAGAGCTCTA GAGAGCTTGC ATGCCTGCAG GTCG
```

Figure 47



Concentration of Expressed Marker Gene "Green Fluorescent Protein" (GFP) versus "Bovine Serum Albumin" (BSA)

Figure 48

Figure 48a

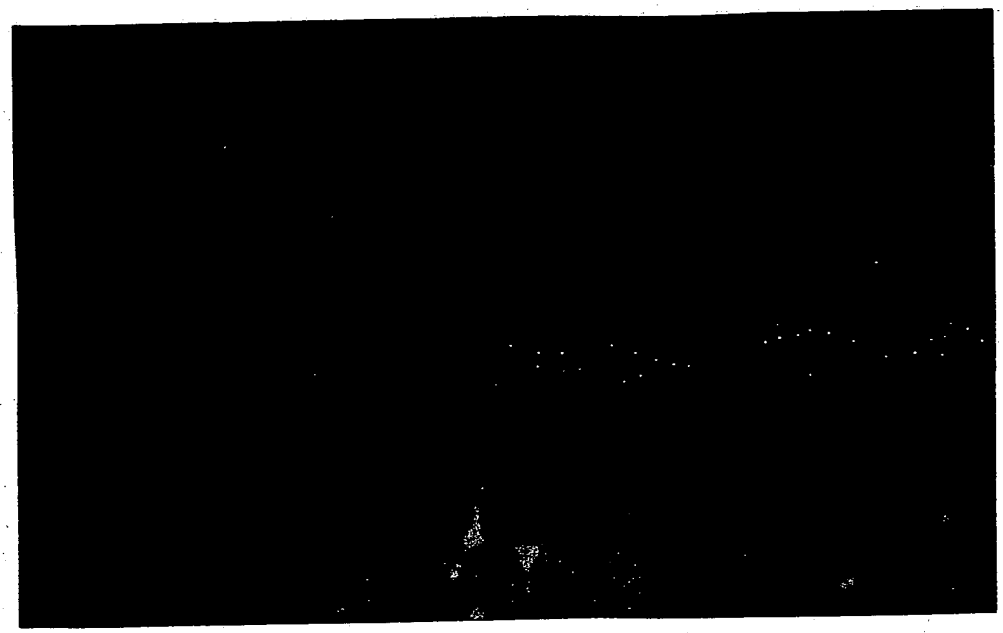


Figure 48b

Figure 48c

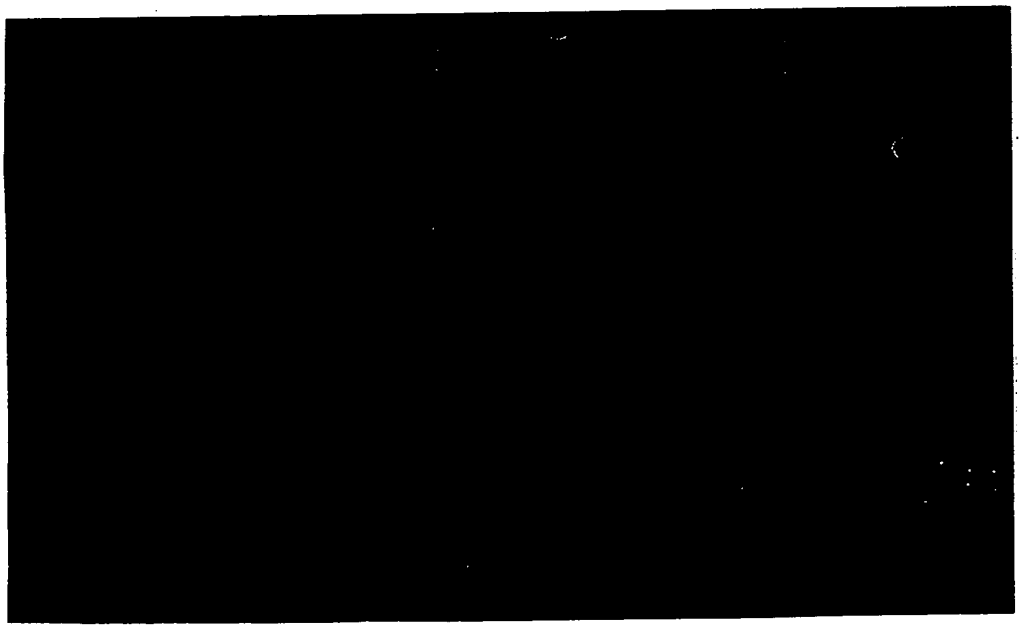


Figure 48d

Corresponding light (a and c) and fluorescent (b and d) microscopic views of HeLa cells transfected with pTGFG5 (a and b) and pTGFG20 (c and d) respectively.